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Fellowships in Medical Oncology: Providence Hospital
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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1980.

GUEST EDITORIAL

Limited Treatment in the Management of Early Breast Cancer[1]

J. L. Hayward[2,*]

The feasibility of conservative therapy for early breast cancer has frequently been questioned in the past 25 years. Conservation of the breast has obvious appeal, not only for the cosmetic advantage, but also because the avoidance of mutilating surgery might encourage women to seek treatment earlier. If early diagnosis and treatment increase the possibility of cure, then the offer of conservative surgery might actually improve survival and recurrence rates.

A number of different treatments have been suggested. The most widely tested treatment has been the use of minimal local surgery—such as wide excision—with radiotherapy to the ipsilateral lymph nodes and breast. Mustakallio (1) in Finland was the first to report an uncontrolled series which suggested that such a treatment could be successful. Crile (2,3) has spent much of his life championing this cause and has argued persuasively in favor of minimal surgery. In Great Britain, a clinical trial was carried out to compare the operation of radical mastectomy with that of wide excision, and the preliminary results suggested that wide excision plus regional radiotherapy might be an acceptable option for clinical stage I tumors, providing a higher rate of local recurrence can be accepted (4). Axillary node recurrence is the major problem, and at least two trials are now underway to test the use of wide local excision with axillary node clearance.

Harris and his colleagues (5) have reported in this issue a short series in which patients with early breast cancer have been treated by radiotherapy alone. The short-term results look encouraging and the question must be raised again as to whether some form of conservative treatment can safely be offered as an option to women with early disease. Each physician will have to decide in his own mind whether he believes this to be so but, in making this decision, the following code might be considered.

(*a*) No unorthodox treatment should be practiced until it has been *proved* to be equal to or better than orthodox treatment—scientifically, this means that a new treatment must have been tested adequately by a clinical trial.

(*b*) If the results of a clinical trial suggest a major change in treatment policy, this clinical trial should be repeated—the results of one trial should not radically affect treatment policy without confirmation.

(*c*) The treatment advised for any patient must be the minimum to give the best possible results.

The question is, can conservative treatment pass these tests? The answer must certainly be no. The results of the treatment of stage I patients by wide excision and radiotherapy have not yet been confirmed, although a new trial has been completed and the results are awaited. The use of wide excision plus axillary node dissection is also still under evaluation and no recommendation for its use can be made until the results of such investigations are available. As far as radiotherapy alone is concerned, its use has never been scientifically tested.

What can be said, with a reasonable degree of certainty, is that some form of conservative therapy is adequate treatment for a number of patients with early disease. The problem is that, at the moment, there is no way to identify those patients for whom such therapy is safe. This is particularly true in view of the established prognostic significance of axillary lymph node involvement. It is ironic that at a time when so many investigators are exploring more limited approaches to the local management of breast cancer, the success of adjuvant chemotherapy has made knowledge of axillary lymph node status mandatory in selecting patients for therapy. Additional nonsurgical ways of identifying patients with microscopically disseminated disease are badly needed. Until we can identify those most suitable for limited approaches, our advice to individual patients must continue to be that conservative treatment carries with it an increased risk of both recurrence and early death. We are beginning to know what that risk is, and it may well be that some patients may opt to take it. This is for them to decide. But our duty as physicians must be to continue to advise our patients to accept therapy, the results of which we know. It is also our duty to

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contribute as much as possible to those scientific endeavors which are now being undertaken to test the validity of other and more conservative options.

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Results of Treating Stage I and II Carcinoma of the Breast With Primary Radiation Therapy [1]

Jay R. Harris,* Martin B. Levene, and Samuel Hellman [2]

SUMMARY

Eighty patients with stage I and II carcinoma of the breast were treated with primary radiation therapy between July 1968 and December 1975 at the Joint Center for Radiation Therapy. The median followup time was 41 months. Only one local recurrence was observed. The survival at 5 years was 88% for stage I and 72% for stage II. The relapse-free survival at 5 years was 91% for stage I and 56% for stage II. Of breasts evaluated for cosmetic results, 66% were judged good or excellent by physicians and 81% were judged good or excellent by patients. One patient developed a pericardial reaction related to treatment which required hospitalization. Other complications were mild and cleared without additional therapy.

[Cancer Treat Rep 62:985–991, 1978]

Radical surgery has been the standard treatment for early carcinoma of the breast in this country. Primary radiation therapy for breast cancer, used more commonly in other countries (1–4), has been reserved either for patients medically unfit for surgery or for women who refused mastectomy. This early experience with radiation therapy has given promising results (5–7) and has encouraged wider use of this form of treatment. At this time, the relative merits and indications for surgery and radiation therapy have yet to be determined.

Surgery and radiation therapy are both forms of local treatment for primary cancer of the breast and its adjacent lymph node areas. It has long been recognized, however, that breast cancer frequently disseminates, with metastases usually appearing in the first 5 years after diagnosis (8), and that effective systemic therapy will be required for cure in most cases. The effectiveness of local treatment, then, should be judged by two major criteria: (*a*) local control, the eradication of cancer in the breast, chest wall, and draining lymph node areas; and (*b*) the cosmetic and functional results after treatment.

Radical mastectomy has been shown to be capable of achieving local control in up to 90% of patients with early carcinoma of the breast (9–12). The cosmetic result following this surgery is limited by the extent of the tissue resected. The situation is complicated by the psychologic importance of breast tissue to most women. Radiation therapy after biopsy offers the possibility of both local control of the tumor and breast preservation. Earlier reports have shown good local control results with radiation therapy, but there has not been a quantitative analysis of the cosmetic result following this form of treatment.

From 1968 through 1975, 80 patients with stage I or II carcinoma of the breast were treated with radiation therapy following biopsy at the Joint Center for Radiation Therapy. This report summarizes the results of this treatment, including an evaluation of the cosmetic result in 29 of these patients.

MATERIALS AND METHODS

In all patients, pathology revealed invasive carcinoma. Three of the patients had nonpalpable primary lesions and were included in the T_1 category. Axillary sampling was performed in only eight

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patients. Patients were clinically staged according to UICC criteria:[3]

T_1 = < 2 cm
T_2 = 2–5 cm
T_3 = > 5 cm
T_4 = attached to chest wall or skin
N_0 = no palpable axillary nodes
N_1 = movable axillary nodes thought to be clinically involved
(N_{1a} axillary node enlargement thought to be benign was considered as N_0)
N_2 = axillary nodes fixed to one another or other structures
N_3 = supraclavicular or infraclavicular nodes or edema of the arm
M_0 = no evidence of distant metastasis
M_1 = distant metastasis present

Stage I = $T_1N_0M_0$ (27 patients)
Stage II = $T_2N_0M_0$ (34 patients)
$T_1N_{1b}M_0$ (seven patients)
$T_2N_{1b}M_0$ (12 patients)

The patients were referred for treatment for two principal reasons: patients had refused mastectomy or were not considered medically fit for surgery. The median age of all patients was 58 years with a range of 27–93 years. Fifty-six patients were > 50 years old while 24 patients were ≤ 50 years old. Only nine patients received adjuvant systemic therapy: three received single-agent chemotherapy, four received combination chemotherapy, one received oophorectomy, and one received oophorectomy and chemotherapy.

At followup, patients were scored for the presence of local recurrence and distant metastases. Any patient with reappearance of tumor in the irradiated breast, chest wall, axillary nodes, or supraclavicular nodal areas was scored as having a local recurrence. The median followup time for surviving patients was 41 months with a range of 13–88 months. Three patients were lost to followup and have been included to the time of the last visit.

In order to determine the cosmetic result following treatment, all patients who were alive without disease were considered eligible for a cosmetic evaluation. Twenty-nine of 46 eligible patients (63%) were evaluated. The only reason for exclusion from the assessment was the inability of the patient to be seen by the authors during the evaluation period. These patients were all seen by at least one of us, were photographed, and answered a questionnaire assessing their own evaluation of the cosmetic result. At the time of evaluation, possible adverse cosmetic effects of treatment were independently scored by us on a 4-point scale. All photographs were reviewed jointly by us and an overall cosmetic score was given: 1 = excellent, treated breast nearly identical to untreated breast; 2 = good, treated breast slightly different from untreated breast; 3 = fair, treated breast clearly different from untreated breast but not seriously distorted; and 4 = poor, treated breast seriously distorted. Figure 1 shows examples of treated breasts scored as excellent and fair.

The details of therapy have been previously described (6). Four of the 80 patients had bilateral cancers treated by definitive radiation therapy. Two of these second cancers were synchronous and two were metachronous. Of the 84 breast cancers treated, 73 underwent excisional biopsy with removal of all gross disease (87%) and 11 had incisional or needle biopsies (13%). Treatment was delivered by megavoltage apparatus at 1000 rads/week in four or five fractions. Opposed tangential fields were used to treat the breast and underlying chest wall and an anterior field was used to treat the ipsilateral axillary and supraclavicular lymph nodes. The total dose was usually 5000 rads with a range of 4000–6300 rads. In 18 patients, an interstitial implant using either ^{192}Ir or ^{226}Ra was performed. In general, patients who failed to undergo excisional biopsy underwent an implant to boost the dose locally in the area of primary cancer.

Survival and relapse-free survival were calculated by the life-table method (13).

RESULTS

Eighty patients with stage I or II carcinoma of the breast were treated by definitive radiation therapy following biopsy during the study. Four of the 80 patients had a bilateral cancer treated for a total of 84 breasts treated by radiation therapy. Figure 2 shows the actuarial likelihood of local control. None of the 28 treated stage I cancers recurred locally and only one of the 56 treated stage II cancers recurred locally. The one local recurrence occurred 8 months following therapy.

Figure 3 shows the actuarial survival for stage I and II patients. At 5 years, the survival was 88% for stage I patients and 72% for stage II patients. Since patients with metastatic breast cancer often survive for long periods of time following relapse, 5-year survival figures for breast cancer give a falsely high estimate of the ultimate survival. Relapse-free survival which deletes patients at the time of relapse is a more reliable index of the ultimate survival (fig 4). The 5-year relapse-free survival was 91% for stage I patients and 56% for stage II

[3]TMN Classification of Malignant Tumors. Union Internationale Contre le Cancer, Geneva, Switzerland, 1974.

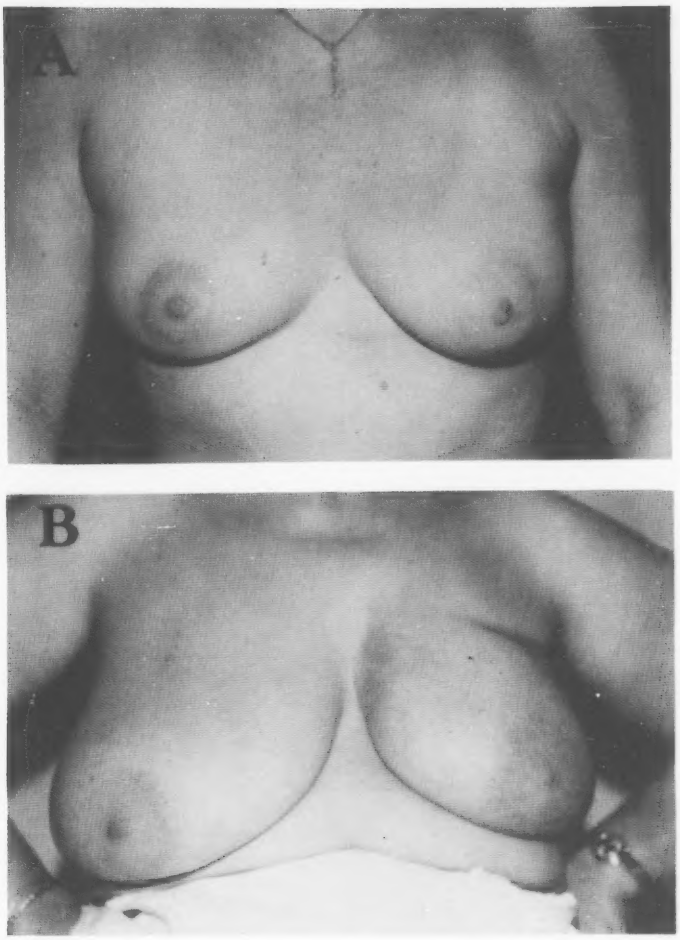

FIGURE 1.—Cosmetic results following radiation therapy in patients given excellent (A) and fair (B) evaluation scores. Both patients presented with T_1N_0 carcinomas of the left breast and underwent excisional biopsies. Patient A received 5600 rads in 28 fractions over 40 days and was photographed 2 yrs and 4 mos after treatment. Patient B received 6000 rads in 25 fractions over 57 days and was photographed 5 yrs and 5 mos after treatment.

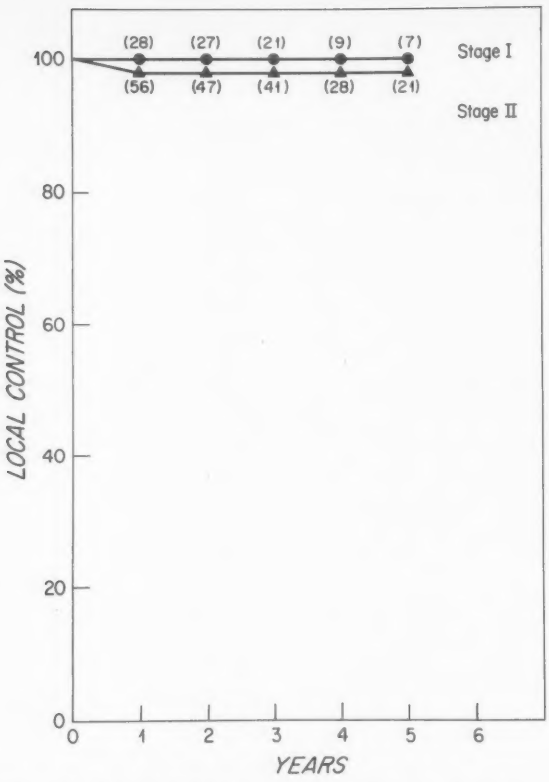

FIGURE 2.—Actuarial likelihood of local control for treated stage I and II carcinomas of the breast. Nos. in parentheses = Nos. of treated breasts followed to given time interval.

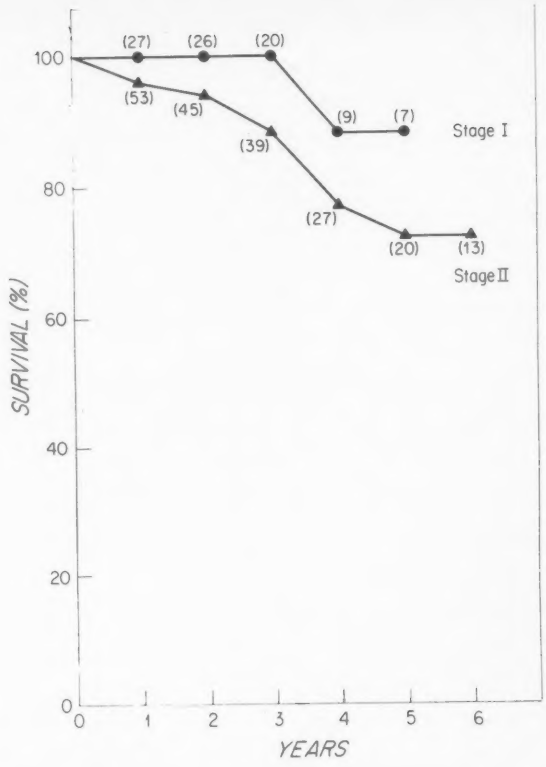

FIGURE 3.—Actuarial survival for patients with stage I and II carcinomas of the breast treated by radiation therapy.

patients. Since only one patient had local recurrence, these relapses reflect the appearance of metastatic disease in these patients.

An assessment of the cosmetic result following therapy was performed on 29 patients. Two of these patients had bilateral breast cancers treated for a total of 31 breasts evaluated. Table 1 shows the results of this assessment. Sixty-six percent of the treated breasts were judged good or excellent by physician evaluation while 81% of the treated breasts were judged good or excellent by patient evaluation. Using a numerical scale of 1 = excellent, 2 = good, 3 = fair, and 4 = poor, the weighted average was 2.1 by physician evaluation and 1.8 by patient evaluation.

In order to determine whether the cosmetic result changed with increasing followup time, the cosmetic results in patients whose treatment conformed to the current guideline for dose schedule were analyzed separately. This guideline is total external beam dose not > 5600 rads given at 200 rads/treatment day. The cosmetic results by physician evaluation were then related to years of followup (table 2). In this group of similarly treated patients, no significant change in the average cosmetic result was noted with followup times varying from 1 to 4 years.

The side effects of this treatment have been previously described (6). Acute reactions are regularly seen at the end of treatment and are restricted to local skin changes. These changes are transient and vary from dry desquamation and hyperpigmentation to areas of moist desquamation. Late reactions in the irradiated volume can occur in the months following treatment (table 3). The most common reaction was rib fractures which occurred in eight of the 84 irradiated volumes (10%). Some of these patients complained of transient chest wall discomfort and roentgenograms revealed rib fractures while others had asymptomatic fractures noted only on routine followup chest roentgenograms; all of the fractures healed without further

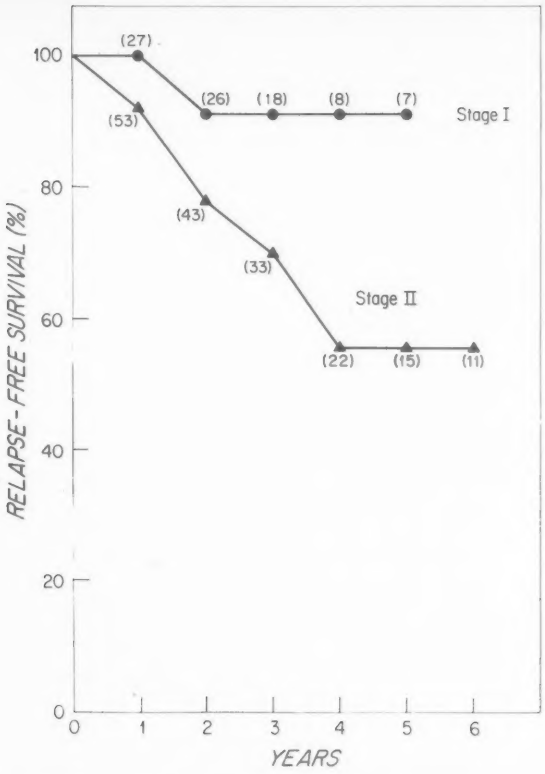

FIGURE 4.—Actuarial relapse-free survival for patients treated by radiation therapy.

treatment. Three patients had radiation-related pulmonary reactions consisting of unproductive cough and mild shortness of breath. In all cases, these symptoms were self-limited and did not require hospitalization. One patient developed a transient ipsilateral pleural effusion 4 months after treatment. No malignant cells were found on thoracocentesis and the effusion cleared spontaneously. The patient is now alive without relapse 2½ years following therapy. The only treatment-related hospitalization occurred in a patient who presented with synchronous bilateral breast cancers. The patient was treated with a nonstandard field arrangement which included an anterior field to treat the internal mammary nodes bilaterally. This patient developed radiation-related pericarditis and eventually required a thoracotomy for creation of a pericardial window.

TABLE 1.—Cosmetic evaluations in 31 breasts following treatment with radiation therapy

	No. of evaluations			
	Excellent	Good	Fair	Poor
Physician evaluation	11	10	7	3
Patient evaluation	12	14	4	1

TABLE 2.—Cosmetic scores related to length of followup

	Followup (yrs)		
	1–2	2–3	3–4
Average cosmetic score*	1.43	1.50	1.50
No. of patients	7	8	4

*1 = excellent, 2 = good, 3 = fair, 4 = poor.

TABLE 3.—Complications of radiation therapy

Complications	No. of patients
Minor (no hospitalization or surgery required)	
Rib fractures	8
Pulmonary reaction	3
Pleural effusion	1
	12 (16%)
Major	
Pericardial reaction	1 (1%)
Total	13 (17%)

DISCUSSION

Surgery and radiation therapy are both forms of local cancer treatment. The natural history of breast cancer is such that, even after the primary tumor is removed, the remainder of the breast, chest wall, and draining axillary nodes are at risk for harboring microscopic deposits of cancer. Therefore, both surgery and radiation therapy are directed to eradicating cancer in these areas. While surgery can be varied by the amount of tissue resected, radiation therapy can be varied by both the tissues to be treated and by the dose of radiation given. The concept of dose response is central to the current practice of radiation therapy. Increasing doses of radiation give increasing likelihood of tumor eradication, but also of local complications. The balance between tumor control and complications depends on many factors. These include the particular normal tissue being irradiated, the tumor cell type, the volume of tissue irradiated, the fractionation schedule, and the bulk of tumor being treated. The importance of this last factor, tumor bulk, has been examined for breast cancer. Doses of 4500–5000 rads have been found to be effective in eradicating microscopic deposits of

breast cancer (14,15), while doses as high as 7000–8000 rads are required for eradicating bulky tumors. The general treatment plan outlined in this paper is for the combination of total excisional biopsy of the primary tumor followed by radiation therapy. Since only microscopic deposits remain after total excisional biopsy, a dose of 5000 rads is effective for local tumor control. Furthermore, 5000 rads is a dose level which has been found to be compatible with a good cosmetic result. For those patients who do not undergo excisional biopsy, an additional dose to the primary tumor is required. This is best achieved by means of an interstitial implant, which maximally spares nearby normal tissues (7).

The results presented here demonstrate that biopsy followed by radiation therapy is an effective means to obtain local tumor control and still maintain a good cosmetic result. The overall local control rate of 99% in this series compares favorably to the 75%–90% control rate reported for surgical series of similarly staged patients (9–11). These results, however, must be viewed as preliminary because of the relatively short followup period. The median followup of 41 months in this study is significantly shorter than the 10-year followup often reported in the surgical literature. Nevertheless, there is good reason to believe that the local control rate at 10 years will still be comparable to the rate obtained in surgical series. Review of reported radiation therapy results reveals that the majority of local control failures occur during the first 2–3 years of followup. Following definitive radiation therapy for stage III breast cancers, 89% of local failures occurred by 2 years (6). In patients treated after mastectomy for breast cancer, 83% of local failures occurred by 2 years (15). In the British trial of radiation therapy in early breast cancer which used doses considered low by current standards (2500–2700 rads to the axilla and 3500–3800 rads to the breast), most local failures occurred by 3 years (16). The relatively short followup in this series has more bearing on the survival rate, since patients with recurrent cancer of the breast frequently live for long periods of time. We have attempted to correct for this by the use of relapse-free survival, which deletes patients at the time of relapse, not death. Our 5-year relapse-free survival rates of 91% for stage I and 56% for stage II are comparable to those for surgically treated patients although differences in patient selection, staging criteria, and statistical analysis make strict comparisons impossible (9–12).

The combination of excisional biopsy and radiation therapy requires expertise on the part of both the surgeon and radiation therapist if a good result is to be achieved. The surgeon should remove gross tumor without doing a wide local excision. In some patients reported here, the cosmetic result was diminished by an excessively wide biopsy procedure. In general, patients who underwent circumareolar incisions had better cosmetic results than patients with more obvious incisions. Surgery may also be required for axillary node sampling to determine the need for chemotherapy. In this area, too, small incisions located as unobtrusively as possible and the avoidance of excessive dissection are recommended.

The requirements for radiation therapy include megavoltage equipment, treatment planning to ensure coverage of all potentially involved areas with maximal sparing of normal tissue and avoidance of overlapping fields, treatment wedges to reduce dose inhomogeneity, the avoidance of bolus, and external beam doses not to exceed 5600 rads given at 200 rads/treatment day. As in any complex medical or surgical procedure, experience and an adequate case load are essential for maximizing results.

For many women, mastectomy is a harsh emotional and physical experience. The combination of biopsy and radiation therapy offers the possibility of both local control of the tumor and preservation of the breast. The preliminary results given here provide support for this approach in early carcinoma of the breast.

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Chemotherapeutic Management of Carcinoma of the Bilharzial Bladder: A Phase II Trial With Hexamethylmelamine and VM-26 [1]

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Carcinoma of the bilharzial bladder is the most common form of cancer in Egypt, especially in men (1). It accounts for 27.6% of all cancer cases reporting to the Cairo Cancer Institute (2) and occurs in a relatively young age group with an incidence peak in the fifth decade of life. The tumor arises from the different bladder walls and rarely from the trigone (3). Multiple tumors are encountered in 25% of patients. Mucosal changes are always present in the form of squamous metaplasia, cystitis glandularis, dysplasia, and carcinoma in situ. The squamous cell type is found in 75% of patients, transitional cell type in 20%, and adenocarcinoma in 5%. The well and moderately differentiated types account for 80% of the squamous cell type, whereas the poorly differentiated type accounts for 65% of the transitional cell variety (1). The disease is generally preceded by and associated with chronic bilharzial cystitis, and there is always a heavy degree of pyogenic bacterial infection.

Radical cystectomy has been the most widely used therapeutic modality for carcinoma of the bilharzial bladder (4). It carries an operative death rate of 10%–15% and offers a 5-year survival rate of 25%–35% (5,6). However, there is always a high incidence of pelvic recurrence because most of the patients reporting for treatment are already in advanced T_3 or T_4 stage (7). The radioresponsiveness of cancer of the bilharzial bladder is generally unsatisfactory (8). Relatively few studies of chemotherapy have been conducted in bladder cancer and none in the squamous cell type. In general, the number of patients has been small and randomized trials have rarely been adopted.

The present study is a single-agent phase II trial for chemotherapeutic management of bilharzial bladder cancer using hexamethylmelamine (HMM) and VM-26. HMM was selected because of its activity against other epithelial tumors and suggestions of efficacy (three objective responses among 11 patients) in bladder cancer in preliminary published reports (9). Its relative lack of myelosuppression was also deemed advantageous in a population likely to include patients with extensive prior irradiation. On the other hand, the choice of VM-26 was predicated on the promising results of its systemic use as a single agent (10–12) and in combination with other drugs (13) in phase II studies of bladder cancer. Its efficacy as adjuvant local therapy has also been compared to that of thioTEPA in T_1 bladder carcinoma after transurethral resection (14). The inclusion of VM-26 in the trial also allowed comparison of response to chemotherapy in patients with bilharzial carcinoma relative to the usual bladder cancer of the transitional cell type.

MATERIALS AND METHODS

Between May and December 1976, 50 patients with advanced bladder cancer were included in this study. Prior to therapy, all of the patients had measurable lesions by clinical, cystoscopic, and radiologic examination. Bidimensional measurements were recorded and all patients had a life expectancy of at least 8 weeks. Patients included in the trial had one of the following characteristics: (*a*) T_3 or T_4 tumor not amenable to radical surgery; (*b*) residual cancer after surgery; (*c*) recurrent tumor after surgery; and (*d*) distant lymph node or hematogenous metastases. The study parameters included physical examination, performance status according to the Karnofsky scale, hemogram, blood urea, serum creatinine, urinalysis, urine cytology, cystoscopy with biopsy, chest roentgenogram, iv urography, and cystography; the hemogram, blood urea, and urine cytology were repeated weekly, while iv urography and cystography were performed monthly.

Response was evaluated by clinical examination every 2 weeks, by cytologic examination weekly, and by radiologic examination monthly. Bidimensional tumor measurements were made and serially recorded and compared with pretreatment measure-

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ments. *Objective partial response* (PR) was defined as a decrease of ≥ 50% in the product of two diameters and a minimum duration of response of 4 weeks. Transient responses of < 4 weeks were not included. *Improvement* was considered a diminution of ≥ 25% but < 50% in the product of two diameters with a minimum duration of 4 weeks. *No response* included patients showing no change or progressive increase in tumor size. Changes in performance status, as well as amelioration of pretreatment symptoms, were also noted and recorded. In addition, repeated cytologic examinations of previously positive urine specimens were carried out. Subjective changes and cytologic alterations alone were not used to define objective responses. Criteria for excluding patients from study included a BUN > 40 mg/100 ml, serum creatinine > 2 mg/100 ml, wbc count < 4000/mm^3, and platelet count < 100,000/mm^3 prior to the treatment.

The patients were randomly allocated to receive HMM or VM-26. HMM was given in a dose of 8 mg/kg/day and was continued indefinitely unless intractable intolerance developed. The total daily dose was divided and given orally after meals. Interruptions for gastrointestinal intolerance were permitted and the drug was reinstituted gradually when symptoms had cleared. VM-26 was given in a dose of 70–100 mg/m^2 as a 60–90-minute iv infusion once per week.

TABLE 1.—Results of HMM treatment in advanced bilharzial bladder cancer

Total No. of patients	26
Age range (yrs)	18–65
Sex	
Women	2
Men	24
No. of patients with—	
Pathology	
Squamous	8
Transitional	15
Anaplastic	3
Prior treatment	
Surgery	9
Partial cystectomy	2
Radical cystectomy	7*
Radiotherapy	2
None	16
Clinical status	
Residual disease	4
Recurrent disease	4
Locally advanced	18
Response	
CR	0
PR	10 (38.4%)
Improvement	12
None	4

*Performed in 1 patient following radiotherapy.

RESULTS

Group I (HMM)

Results obtained with HMM treatment of 26 patients are outlined in table 1. The drug caused nausea and vomiting in all patients but this was partially relieved by antiemetics of the phenothiazine group and by temporarily withholding the drug. Within 2–3 weeks, the drug generally became tolerable and antiemetics could be stopped.

Ten of the patients achieved PR, including six with the transitional cell type, one with anaplastic cell type, and three with squamous cell carcinoma. Patients achieving an objective response always exhibited some reduction of symptoms as well. Tumor regression was noted in all of the patients within 2–4 weeks after initiating therapy and the median duration of response was 4 months. However, two patients, both of whom had transitional cell tumors, underwent radical cystectomy after 30 and 35 days, respectively, of HMM treatment. These patients are still being followed and are without disease recurrence 12 and 13 months after surgery. None of the other patients achieved a complete response (CR) in spite of continuing treatment. Two other patients with transitional cell carcinoma are still receiving HMM with PRs occurring 10 and 15 months after the onset of therapy. Both patients have refused surgical intervention. In the remaining six patients who experienced PRs, relapse occurred within 3–6 months and HMM was stopped. Except for the two patients who underwent surgery and the two still receiving treatment, the other patients died from 6 to 9 months after the onset of HMM therapy.

There was objective improvement, generally associated with subjective benefit, in 12 patients, including seven with the transitional cell type, two with anaplastic cell type, and three with squamous cell carcinoma. Survivals in these 12 patients ranged from 4 to 7 months. In nine of these patients, the reduction in tumor size was maintained for 2–4 months before relapse. In the other three patients, the improvement was very brief and relapse was manifest after 1 month of treatment. Four patients, two with transitional cell and two with squamous cell carcinoma, showed no response and died within 2 months after the onset of treatment.

Followup cytologic smears showed cellular evidence of chemotherapeutic effect in all the responding patients (22 of 26). In squamous cell carcinoma,

actual cell necrosis and sloughing were prominent features, especially in the malignant keratinized cell clumps containing pearls and in the shedded single cells as well. Swelling of the cytoplasm and nucleus, without much change in the ratio of nucleus to cytoplasm, was seen in both the squamous and transitional cell types. Nuclear hyperchromasia with loss of chromatin sharpness, increase in the molecular size, and, occasionally, multinucleation were noticed. Some degree of degeneration was present in both the cytoplasm and nucleus in the form of fine and coarse vacuolization with increased density of the cytoplasm surrounding the vacuoles. Since these changes have not been observed during serial observations for other reasons, they are perhaps attributable to cytotoxic effects of HMM. Numerous inflammatory and necrotic cells were seen because all patients were suffering from urinary pyogenic infection.

Gastrointestinal toxic effects were present in all patients but the drug could still be maintained with occasional interruptions. The wbc count was high at the start of therapy, ranging from 10,000 to 20,000/mm^3 in all patients. Followup determinations showed an absence of anemia, leukopenia, and thrombocytopenia. The lowest total wbc count was 4000/mm^3.

TABLE 2.—Results of VM-26 treatment in advanced bilharzial bladder cancer

Total No. of patients	24
Age range (yrs)	30–69
Sex	
Women	2
Men	22
No. of patients with—	
Pathology	
Squamous	9
Transitional	11
Mixed squamous and transitional	1
Anaplastic	3
Prior treatment	
Surgery	9
Partial cystectomy	5
Radical cystectomy	3
Simple diversion	1
Radiotherapy	1*
None	15
Clinical status	
Residual disease	2
Recurrent disease	6
Locally advanced	16
Response	
CR	0
PR	1 (4.2%)
None	23

*Performed following surgery.

Group II (VM-26)

Twenty-four patients were treated with VM-26 and the results are summarized in table 2. Ten patients were treated at a dose of 70 mg/m^2/week by iv drip. No subjective or objective response was noted and the tumors kept increasing in size. The general condition, as judged by performance status, deteriorated rapidly in many of these patients with progressive disease. Drug therapy was stopped in all of these patients after four to six doses. Mild nausea and vomiting occurred in three patients but was not accompanied by myelosuppression. Five patients died within 6 weeks and the other five died within 3 months after initiating therapy.

In the subsequent 14 patients, the dose was increased to 100 mg/m^2/week, with the patients receiving from four to six doses of the drug. Only one patient (mixed squamous and transitional cell carcinoma type) achieved a PR. Subsequently, he underwent cystectomy after six doses of VM-26 and is still alive (12 months) with no disease recurrence and is in good general condition. None of the other patients exhibited any response and their tumors measurably progressed. Again, the survival of these 14 patients was very short and consistent with other patients with progressive disease; death occurred in all patients within 3 months. At this dose, hematologic effects were more prominent; three of 14 patients showed anemia (decline of > 2 g of hemoglobin), seven of 14 showed grade 2 leukopenia (2000–3000 cells/mm^3), and none had thrombocytopenia. Diarrhea, manifested by five to six bowel movements per day, occurred in seven patients (in one of the patients it was very severe and was considered to have contributed to death which occurred within a few days after drug toxicity appeared).

Cytologic examination in patients with squamous cell carcinoma revealed few of the cytologic changes described above. Heavy excretion of necrotic cells was not encountered. No cytologic changes were detected in transitional cell types.

DISCUSSION

This study shows that HMM has a chemotherapeutic effect on cancer of the bilharzial bladder, including both transitional and squamous cell types. This drug yielded an overall PR rate of 38.4%, which was manifested clinically by subjective benefit and suggestion of prolonged survival in responders. In fact, two patients eventually were able

to undergo cystectomy and are currently free of disease, and two other patients have PRs while still receiving the drug. The side effects of HMM were minimal, although nausea and vomiting precluded uninterrupted administration of the drug. Neither neurologic complications nor myelosuppression was recorded.

These promising results encouraged us to continue HMM at higher doses in bladder cancer patients. Doses of 700 mg/m²/day × 7, a rest for 2 weeks, and a repeat course were instituted in a new group of seven patients. Unfortunately, none of the patients tolerated this dose because of severe intractable nausea and vomiting. We expect to initiate further trials of HMM in combination with other agents which are determined to be active in the future or to await the introduction of a new HMM derivative, pentamethylmelamine, which can be given iv.

On the other hand, the role of VM-26 in the chemotherapeutic management of cancer of the bilharzial bladder appears limited. Only one PR was noted among 24 patients. Although ten of the patients received lower doses of VM-26, the toxicity of the high-dose regimen was considerable. Thus, our results in bilharzial cancer differ from those obtained in nonbilharzial bladder cancer. For example, one of the clinical screening trials of the European Organization for Research on Treatment of Cancer (10) achieved one CR and four PRs among 24 patients, for an overall response rate of 20.8%. In another trial, Pavone-Macaluso et al (11) reported two CRs and three PRs in 30 patients, for an overall response rate of 16.6%. Rodriguez et al (13), using a combination of VM-26 and adriamycin, found five PRs in 27 patients (19%).

Explanations for the lack of activity of VM-26 in our study are speculative. However, noteworthy differences in tumor vascularity and tumor cell kinetics may exist as compared to other bladder cancers. Also, hematologic and gastrointestinal tolerance to the agent was poor in our experience with the higher doses of VM-26. In only one instance was this intolerance possibly related to prior treatment with radiotherapy. Delayed excretion of the drug associated with deranged renal function, which is commonly present, might be another explanation. This occurrence, coupled with the peculiar etiology and histology of this cancer, suggests that the findings of phase II studies in bilharzial bladder cancer should not be readily extrapolated to other bladder cancers, and vice versa.

Nevertheless, HMM would appear to be worthy of trial in other forms of bladder carcinoma unassociated with bilharziasis. This agent, or its analogs, will certainly find a role in combination with other forms of therapy in future trials dealing with bilharzial bladder cancer.

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Lack of Therapeutic Synergism Between Vincristine and Methotrexate in L1210 Murine Leukemia In Vivo [1]

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SUMMARY

In vivo studies were performed in mice bearing L1210 ascites tumor to examine the interaction of vincristine (VCR) at doses of 0.15 and 1.5 mg/kg with methotrexate (MTX) at doses of 0.5, 5, 50, and 350 mg/kg. The combination was administered on Days 2, 6, and 10 after tumor inoculation. VCR was given either concurrently with MTX or preceding it by 30, 60, or 120 minutes. VCR administered at a dose of 0.15 mg/kg achieved a peak peritoneal fluid concentration of 1.4 μM declining with a half-life ($T_{\frac{1}{2}}$) of 33.5 minutes and produced no in vivo augmentation of MTX uptake. VCR administered at a dose of 1.5 mg/kg achieved a peak peritoneal fluid concentration of 16.5 μM declining with a $T_{\frac{1}{2}}$ of 24.9 minutes and produced significant in vivo augmentation of MTX uptake when given concurrently with the MTX and 30 minutes prior to MTX at a dose of 350 mg/kg. No other interval was associated with augmented MTX uptake. When animal survival was evaluated, no therapeutic synergism was observed at any dose level of either drug in any schedule. In fact, VCR (0.15 and 1.5 mg/kg) and MTX at doses of $\geq$ 50 mg/kg produced toxic synergism which adversely affected survival. Since the drug doses studied are comparable with those used in "high-dose" clinical protocols, individual tumor evaluation is indicated to support the use of these drugs in combination.

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The efficacy of methotrexate (MTX) in the treatment of a variety of neoplasms has been appreciated for some time (1). Clinical studies suggesting the activity of "high-dose" MTX with citrovorum factor rescue for drug-resistant hematologic and solid tumors (2,3) have occasioned renewed investigation of its pharmacology. Recent work has shown that MTX enters mammalian tumor cells by a carrier-mediated transport mechanism and that saturation of its target enzyme, dihydrofolate reductase, is necessary for inhibition of DNA synthesis (4,5). The observations that vincristine (VCR), another antineoplastic agent, enhances the cellular uptake of MTX in animal (6,7) and human (8) tumor cells and appears to augment MTX's inhibition of deoxyuridine incorporation into DNA in Ehrlich ascites tumor cells in vitro (9) have led to its inclusion in clinical protocols using "high-dose" MTX (10). However, with the exception of the observations of Zager et al (7), in vivo data to support the therapeutic synergism of this drug combination are lacking. The present investigation was undertaken to examine whether such therapeutic synergism can be demonstrated and whether this effect is dependent on the VCR or MTX dose or on the timing of their administration.

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MATERIALS AND METHODS

Survival Studies

CDF_1 female mice, weighing approximately 20 g each, were inoculated ip with 1×10^5 L1210 ascites tumor cells on Day 0 and separated into cohorts of ten animals each. The bedding was changed every 3

days and food and water were given ad libitum. Beginning on Day 2 and then every 4 days thereafter (a schedule previously shown to be optimal for MTX administration [7]), animals were treated ip with saline alone, VCR alone (0.15 or 1.5 mg/kg), MTX alone (0.5, 5, 50, or 350 mg/kg), or the VCR–MTX combination in a total volume of approximately 200 μl (0.01 ml/g of body weight). Toxicity studies on this mouse strain suggest that 5 mg/kg is the LD10 dose, that 25 mg/kg is the LD50 dose, that 50 mg/kg is the LD90 dose, and that 350 mg/kg is greater than the LD90 dose of MTX. The higher doses were studied principally for transport purposes and not to assess any effect on survival. Combination treatment was administered using four schedules with VCR given simultaneously with MTX (0 time interval) or preceding it by 30, 60, or 120 minutes on each day of treatment. During treatment, cohorts were maintained in separate cages and their survivals were recorded from the day of tumor inoculation. Drugs were obtained in powder form from the Drug Development Branch, Developmental Therapeutics Program, DCT, NCI.

In Vivo Transport Studies

CDF_1 female mice identical to those described in the preceding section were inoculated ip with 1×10^5 L1210 ascites tumor cells on Day 0 and used for study on Day 6 when ascites fluid contamination with red cells was minimal and Trypan blue exclusion exceeded 95%. Using the identical schedule of VCR and MTX administration previously described, animals were treated with ip VCR administered at a dose of 0.15 or 1.5 mg/kg contained in 100 μl of sterile 0.85% NaCl. Control animals received 100 μl of 0.85% NaCl. At the appropriate time interval following VCR or 0.85% NaCl administration, animals were treated with tritium-labeled MTX ip at doses of 0.5, 5, 50, and 350 mg/kg contained in 100 μl of sterile 0.85% NaCl. The tritiated MTX (3′5′9[n]^{3}H–MTX; Amersham Searle Corp, Arlington Heights, Ill) was purified as previously described (4) and diluted with purified, unlabeled MTX to give a final specific activity of 3.7–211 $\times$ 10^6 dpm/μmol. Thirty minutes following the injection of the tritiated MTX, a time sufficient for ascites tumor cells to reach a steady-state for MTX and during which peritoneal fluid MTX concentrations remained relatively constant, animals were killed by cervical dislocation and the L1210 ascites tumor was lavaged out with 4°C bicarbonate-buffered 0.85% NaCl and collected by centrifugation. The cells were washed twice to remove extracellular MTX, and the resulting cell pellet was drawn up into the tip of a Pasteur pipette and gently extruded onto a polyethylene tare. Each pellet contained approximately 5×10^7 tumor cells. Following overnight drying at 70°C, the cell pellet was weighed on a Cahn RG autoelectrobalance with digital readout (Cahn Instruments, Inc, Paramount, Calif). Pellets were placed in the bottom of a scintillation vial and digested in 1 N KOH at 70°C for 1 hour. After cooling to room temperature, 18 ml of a methanol-toluene scintillation fluid (8) was added and the vials were counted in a Searle Mark III liquid scintillation system (Searle Analytic, Inc, Chicago, Ill). The tritium counting efficiency was 26% and the quench variation between samples was negligible. Results were expressed as nanomoles of MTX per gram of dry cell weight. Replicate experiments were performed on three animals on at least 3 different days with duplicate determinations taken at each experimental point.

The nonexchangeable MTX content of L1210 ascites cells was determined under each experimental condition described above. After animals were killed by cervical dislocation, ascites cells were lavaged out with 4°C bicarbonate-buffered 0.85% NaCl and washed twice. The resulting pellet was then resuspended in a glass incubation flask containing a large volume of 37°C Eagle's minimal essential medium without serum or folates. The suspension was continuously agitated by a mechanical stirrer and a stream of warm, humidified 95% O_2–5% CO_2 passed over the suspension. At designated time intervals, samples were removed and processed as described above to determine ^{3}H–MTX content per gram of dry cell weight.

The exchangeable intracellular MTX level was determined by subtracting the noneffluxable intracellular MTX level from the total intracellular MTX under each experimental condition. The extracellular water content was determined as previously described (4) by measuring the ^{14}C-inulin space and subtracting this from the difference between the wet and dry cell pellet weights. This allowed determination of an intracellular water to dry cell pellet weight ratio for each cell line at each MTX concentration which provides an estimate of intracellular volume (4). The ^{14}C-inulin was obtained from the New England Nuclear Corp (Boston, Mass).

MTX Concentrations

The ascites fluid MTX concentrations corresponding to each dose level were determined by injecting Day-6 tumor-bearing animals with 500 μl of purified (4) tritium-labeled MTX in 0.85% NaCl adjusted to appropriate specific activities (3.7–211 $\times$ 10^6 dpm/μmol) with purified, unlabeled MTX. At 15-minute intervals following injection, three animals were

killed by cervical dislocation and duplicate 25-μl aliquots of ascitic fluid obtained from each animal were placed in a methanol-toluene scintillation fluid (8). Samples were counted in a Searle Analytic Mark III scintillation counter with a tritium counting efficiency of 26% and negligible quench variation between samples. This permitted determination of ascites fluid MTX concentrations and comparison of these values with plasma MTX concentrations achieved in man during "high-dose" therapy (11). The plasma concentrations achieved at each MTX dose in tumor-bearing mice have been previously reported (12). However, a direct comparison is not possible because the elimination pharmacokinetics of MTX differ between mouse and man (13).

VCR Concentrations

The ascites fluid VCR concentrations corresponding to each dose level were determined by injecting Day-6 tumor-bearing animals with 500 μl of purified (14) aromatically labeled tritiated VCR prepared by Dr. James Kutney of the University of British Columbia (Canada). The specific activity of the injected material was 40 microcuries/mg. At 1, 5, 10, 20, 40, 60, 90, and 120 minutes after the injection, three animals were killed by cervical dislocation and duplicate 25-μl aliquots were obtained from each animal at both VCR doses. The samples were placed in a methanol-toluene scintillation fluid and processed as described in the preceding section. The VCR doses were chosen because they are equivalent (15) to human doses of 0.45 and 4.5 mg/m^2, which span currently used clinical doses, and also because they achieve sufficiently high ip fluid VCR levels to augment in vitro MTX transport in this L1210 cell line. The results were expressed as a VCR concentration and the data points fit by a least mean squares linear regression analysis to determine the peritoneal fluid VCR half-life ($T_{\frac{1}{2}}$). The data best fit a single exponential decay described by $C_p = ae^{-bt}$ where C_p is the peritoneal fluid VCR concentration as a function of time and a and b are constants determined by regression analysis. The $T_{\frac{1}{2}}$ values at each VCR dose were calculated from the constant b according to the following equation:

$$T_{\frac{1}{2}} = \frac{0.693}{b}.$$

In Vitro Transport Studies

Parallel transport studies were carried out on Day-6 tumor-bearing animals to assess the threshold for the VCR effect on MTX uptake in this L1210 cell line (7). Animals were killed as before and tumor cells were collected by peritoneal lavage and centrifugation at 750 $\times$ g in 4°C bicarbonate-buffered 0.85% NaCl and washed once prior to resuspension in Eagle's minimal essential medium without serum or folates at a cell density of 1–2 $\times$ 10^7 cells/ml. The cell suspensions were placed in glass incubation flasks immersed in a 37°C water bath and the suspensions were continuously agitated by a mechanical stirrer. A stream of warm, humidified 95% O_2–5% CO_2 gas mixture was passed over the suspension for 5 minutes prior to the addition of VCR which achieved bath concentrations of 0.1, 1, and 10 μM or no VCR for control baths. Following a 5-minute preincubation period, ^{3}H–MTX was added to achieve a bath concentration of 1 μM and incubation was continued for 5–60 minutes. Samples (2–4 ml each) were withdrawn at regular intervals, and uptake was terminated by rapid centrifugation and two 4°C 0.85% NaCl washes to remove extracellular MTX. The resulting pellet was drawn up into the tip of a Pasteur pipette and processed as described earlier. Results were expressed as the percent augmentation of total intracellular MTX as compared to simultaneous controls of MTX alone. Replicate experiments were performed on 3 different days with duplicate determinations at each experimental point.

Statistical Analysis

Survival data.—Each group of data (for ten mice) was tested by the method of Epstein (16) and was found to be consistent with a two-parameter exponential survival distribution. As such, the mean time between animal deaths (θ) may be used to describe survival. Estimates of θ were calculated by the multifactorial method of Zelen (17) and were analyzed by his exponential analysis of variance method that allowed for the determination of an MTX effect, a VCR effect, and an effect due to the interaction of the two drugs.

Transport data.—The in vivo transport data were expressed as the mean uptake under each experimental condition and were analyzed by multiple linear regression with analysis of variance tests for deletion of variables (18). The in vitro transport data were analyzed by a mixed-model analysis of variance (19).

RESULTS

Animal Survival Studies

Because both VCR and MTX may have a therapeutic (antitumor) and toxic effect, there were four drug effects of interest in this experiment: (*a*) the effect of MTX alone in prolonging survival; (*b*) the

effect of VCR alone in prolonging survival; (*c*) the synergistic effect of the combination at each time interval in prolonging survival; and (*d*) the synergistic toxic effect of the combination at each time interval in shortening survival. Synergism denotes that the effect of the combination in altering survival was greater than the simple additive effect of the single agents. Animal survival data are contained in table 1. For the 0 time intervals, only MTX prolongs survival ($P < 0.01$), VCR not having a statistically significant beneficial effect ($P = 0.11$) alone or in combination, although there is evidence for toxic synergism at high levels of MTX, ≥ 50 mg/kg ($P < 0.01$). For other schedules, both drugs prolong survival independently at MTX levels of ≤ 5 mg/kg ($P < 0.025$), but continue to cause toxic synergism at higher MTX levels ($P < 0.005$). In no schedule was therapeutic synergism observed at any dose level of either agent.

In Vivo Transport Studies

The results of in vivo transport studies are summarized in table 2. The tenfold concentration increments from 0.5 to 5.0 mg/kg, from 5.0 to 50 mg/kg, and from 50 to 350 mg/kg resulted in 2.7-, 1.6-, and 2.6-fold increments in total intracellular MTX, respectively. VCR at a dose of 0.15 mg/kg did not augment intracellular MTX at any MTX dose or interval of administration. Cellular MTX was significantly ($P < 0.0006$) augmented by 1.5 mg/kg of VCR given simultaneously with MTX at each MTX dose level and at the 30-minute time interval at an MTX dose of 350 mg/kg. An augmentation in total intracellular drug of 29%, 12%, 32%, 28%, and 32%, respectively, was observed under each of these experimental conditions. With those exceptions, 1.5 mg/kg of VCR given at any other time prior to MTX at any dose did not augment intracellular MTX. Moreover, when the nonexchangeable MTX level was measured under each experimental condition, VCR was found not to significantly influence the extent of high-affinity intracellular MTX binding. A nonexchangeable level of 4.36 ± 0.14 mmols/g of dry cell weight (mean ± SE) was recorded for this L1210 cell line. This value is similar to that previously reported by other investigators (5,7) for MTX-sensitive L1210 leukemia cells. By subtracting the nonexchangeable MTX level from the total intracellular MTX, the exchangeable intracellular drug level can be determined (4). If this value is divided by the ratio of intracellular water to dry cell weight, an estimate of the intracellular volume (4), then the free intracellular MTX concentration ($[MTX]_i$) can be determined. The intracellular volumes expressed as ratios of intracellular volume in milliliters per gram of dry cell pellet weight were not influenced by the presence of VCR and were 4.31 ± 0.17, 4.02 ± 0.11, 3.90 ± 0.20, and 3.81 ± 0.26 ml/g for MTX doses of 0.5, 5, 50, and 350 mg/kg, respectively, 30 minutes after MTX administration. The $[MTX]_i$ at each MTX dose with or without concurrent VCR administration at 1.5 mg/kg is summarized in table 3. VCR significantly augments the $[MTX]_i$ at each MTX dose with peritoneal fluid MTX concentrations ranging from 2.8 to 2062 μM when administered concurrently.

Ascites Fluid MTX Concentrations

The ascites fluid MTX concentrations achieved at each MTX dose level were measured 15–30 minutes

TABLE 1.—Mean animal survival time (days ± SD)*

VCR-MTX interval (mins)	VCR dose (mg/kg)	MTX dose (mg/kg)				
		0	0.5	5	50	350
0	0	7.50 ± 0.53	7.90 ± 0.74	10.8 ± 1.54	9.10 ± 2.18	8.10 ± 1.37
	0.15	7.80 ± 0.63	8.80 ± 0.79	12.8 ± 1.69	9.40 ± 1.35	6.00 ± 1.15
	1.5	10.2 ± 0.63	10.1 ± 0.88	13.8 ± 2.14	9.40 ± 3.03	5.60 ± 0.52
30	0.15	7.80 ± 0.63	9.00 ± 0.67	12.1 ± 1.66	11.5 ± 1.64	8.30 ± 2.79
	1.5	10.2 ± 0.63	11.0 ± 1.24	13.5 ± 2.12	7.90 ± 2.02	5.50 ± 0.53
60	0.15	7.80 ± 0.63	8.90 ± 0.74	12.2 ± 1.47	9.00 ± 2.45	7.30 ± 1.25
	1.5	10.2 ± 0.63	10.7 ± 2.26	13.6 ± 1.71	8.20 ± 1.99	5.60 ± 0.52
120	0.15	7.80 ± 0.63	8.20 ± 1.84	11.4 ± 1.84	8.90 ± 1.96	5.80 ± 0.92
	1.5	10.2 ± 0.63	10.7 ± 1.83	11.8 ± 2.09	7.70 ± 2.79	5.50 ± 0.53

*Cohorts of CDF_1 female mice bearing L1210 ascites tumor were treated with saline alone, MTX alone at 0.5, 5, 50, or 350 mg/kg, VCR alone at 0.15 or 1.5 mg/kg, and VCR-MTX combinations on Days 2, 6, and 10 following tumor inoculation and their survival was recorded. VCR dose 0 and MTX dose 0 are saline-only controls. VCR dose 0 and MTX doses of 0.5, 5, 50, and 350 mg/kg are MTX-only controls. MTX dose 0 and VCR doses of 0.15 and 1.5 mg/kg are VCR-only controls.

TABLE 2.—MTX uptake in vivo under each experimental condition*

VCR-MTX interval (mins)	VCR dose (mg/kg)	MTX dose (mg/kg)			
		0.5	5	50	350
0	0	12.09 ± 0.87	33.18 ± 2.10	52.24 ± 2.34	136.50 ± 3.49
	0.15	11.27 ± 1.42	28.05 ± 0.15	50.20 ± 0.94	140.20 ± 1.85
	1.5	16.47 ± 0.23†	36.63 ± 0.69†	71.13 ± 5.57†	176.7 ± 13.94†
30	0.15	11.63 ± 0.23	29.40 ± 1.68	50.68 ± 4.02	136.00 ± 2.05
	1.5	13.83 ± 0.48	32.20 ± 2.73	53.30 ± 1.53	180.80 ± 23.84†
60	0.15	12.85 ± 1.27	29.47 ± 1.18	56.64 ± 4.49	136.17 ± 2.30
	1.5	15.23 ± 1.80	33.80 ± 1.07	52.4 ± 2.13	134.03 ± 20.10
120	0.15	12.47 ± 0.32	28.53 ± 0.62	46.20 ± 0.92	130.67 ± 1.16
	1.5	13.47 ± 0.92	31.97 ± 1.55	52.40 ± 0.98	121.08 ± 2.91

*Cohorts of CDF_1 female mice bearing L1210 ascites tumor were treated with 0.85% NaCl alone or VCR at a dose of 0.15 or 1.5 mg/kg ip followed by MTX at a dose of 0.5, 5, 50, or 350 mg/kg ip at an interval of 120, 60, 30, or 0 mins on Day 6 after tumor inoculation. 30 mins following MTX administration, animals were killed, the ascites tumor was lavaged out, and the total intracellular MTX was determined. Results are expressed in nmols/g of dry cell weight as mean ± SE.

†Given result is statistically different ($P < 0.0006$) from the control.

after ip drug administration, the period during which uptake experiments were performed. For MTX doses of 0.5, 5, 50, and 350 mg/kg, ascites fluid concentrations of 2.80 ± 0.21, 21.0 ± 2.6, 216 ± 39, and 2062 ± 300 μM, respectively, were observed. These data are expressed as means ± SD. If these concentrations are divided into the $[MTX]_i$ determined for each MTX dose level (*see* table 3), then distribution ratios for MTX can be calculated. These are 0.69, 0.32, 0.057, and 0.017 at MTX concentrations of 2.80, 21.0, 216, and 2062 μM, respectively.

Ascites Fluid VCR Concentrations

The ascites fluid VCR concentrations achieved at 0.15 and 1.5 mg/kg doses are depicted graphically in figure 1. At a dose of 1.5 mg/kg of VCR, peak peritoneal fluid concentrations of 16.5 μM were measured which decayed with a $T_{\frac{1}{2}}$ of 24.9 minutes. The concentrations at 30, 60, and 120 minutes were 7.3, 3.3, and 0.61 μM, respectively. At a dose of 0.15 mg/kg of VCR, peak peritoneal fluid concentrations of 1.4 μM were measured which declined with a $T_{\frac{1}{2}}$ of 33.5 minutes. The concentrations at 30, 60, and 120 minutes were 0.70, 0.34, and 0.086 μM, respectively. The data best fit a single exponential decay in contrast to the biphasic or triphasic decay recently described for blood radioactivity in animals (20) and in man (14), respectively.

TABLE 3.—Effect of concurrent VCR administration on the intracellular MTX concentration in vivo*

MTX dose (mg/kg)	$[MTX]_i$ without VCR (μM)	$[MTX]_i$ with VCR (μM)	% augmentation
0.5	1.94	2.79	44
5	6.65	7.60	14
50	12.27	16.57	35
350	34.86	44.72	28

*MTX concentrations corresponding to each dose level will be found in the Results section (Ascites Fluid MTX Concentrations). VCR dose was 1.5 mg/kg in each instance, and VCR and MTX were administered concurrently.

In Vitro Transport Studies

In vitro transport studies were performed to determine whether the L1210 cell line used in this study behaved similarly to that previously described by Zager et al (7) with respect to VCR augmentation of MTX uptake. The results of these studies are summarized in table 4. VCR concentrations of 10 and 1 μM significantly augmented intracellular MTX by 27% and 15%, respectively, but 0.1 μM VCR failed to do so. These data are in agreement with the percent augmentation observed in vivo with concurrently administered VCR at a dose sufficient to reach 10 μM ip (1.5 mg/kg). However, the augmentation observed by Zager et al (7) at 0.1 μM VCR could not be reproduced, whereas the augmentation at higher VCR concentrations could be reproduced, but not to the same extent.

DISCUSSION

The results of our study demonstrate that in a murine tumor system sensitive to MTX and in which VCR augments MTX uptake, the therapeutic synergism of MTX and VCR is not found at MTX

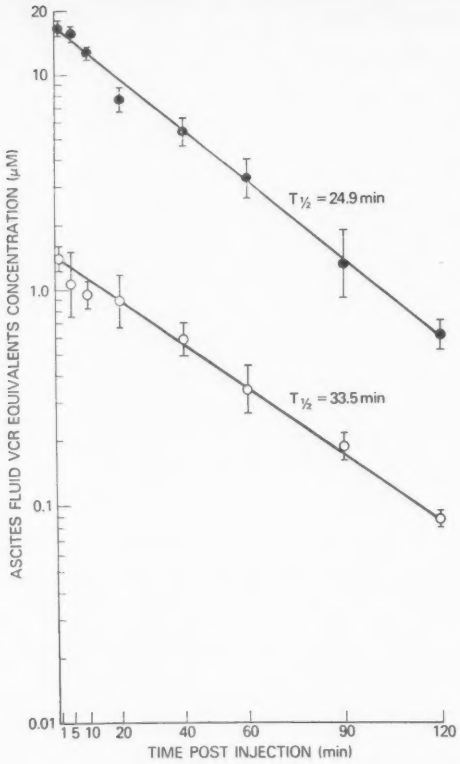

FIGURE 1.—Peritoneal fluid VCR concentrations at 1.5 mg/kg (●) and 0.15 mg/kg (○) in vivo. Mice were inoculated with tritiated drug and peritoneal fluid was sampled at the designated times. Results are expressed as means ± SE.

concentrations comparable to those achieved clinically in "high-dose" protocols (11). More importantly, toxic synergism was noted at MTX doses ≥ 50 mg/kg. These doses give mean ascites fluid MTX concentrations of at least 216 μM, levels achieved in the plasma by "high-dose" infusions in man (11). The disparity between these data and those previously reported by Zager et al (7) may relate to the difference in the MTX dose employed, since the schedule as well as the 1.5-mg/kg VCR dose were identical to the experimental design of this earlier work. However, additional studies performed by us suggest that 5 mg/kg given ip every 4 days is the LD10 dose and that 25 mg/kg is closer to the LD50 dose in this mouse strain. These differences suggest that comparison of toxicity or survival data among different investigators may be unwise. Moreover, the difference between the VCR threshold for augmentation of MTX uptake in vitro observed by us and that reported by Zager et al (7) suggests that some alteration in the L1210 cell line may have also occurred. A second possible explanation is that L1210 strains may differ with respect to the VCR effect. This postulate is supported by the recent work of Chello et al (21) in which the design of the Zager study was repeated. They observed that VCR (0.5 mg/kg) given 30 minutes prior to MTX produced no significant change in intracellular MTX persistence or in augmentation of animal survival compared to MTX alone. These data are in agreement with the in vitro studies of Bruckner et al (22) in L1210 cells in which VCR at concentrations of 1 and 10 μM was observed to decrease the MTX effect on de novo DNA synthesis. In vitro studies in human lymphoblast and myeloblast cells further support the concept of individual tumor thresholds. MTX uptake was augmented in human lymphoblast cells at VCR concentrations of 10 μM but not at 0.1 μM (23), in contrast to the augmentation of MTX uptake observed at both VCR concentrations in human myeloblast cells (8). These differences suggest that individual tumor thresholds for the VCR effect on MTX uptake need to be established before employing the drug combination clinically.

The VCR-induced augmentation of MTX uptake appears to be dependent on at least two variables in vivo, the VCR concentration at the time of MTX administration and its persistence during the period of drug uptake. VCR doses of 0.15 mg/kg did not augment MTX uptake at any interval or at any MTX dose. In vitro studies in these L1210 cells

TABLE 4.—In vitro augmentation of MTX uptake in L1210 leukemia cells*

MTX concentration (μM)	VCR concentration (μM)	Total intracellular MTX (nmols/g of dry cell weight)	% augmentation
1	0	7.99 ± 0.081	—
1	0.1	8.09 ± 0.154	1.2
1	1	9.17 ± 0.200†	15
1	10	10.16 ± 0.751†	27

*Experimental details are described in the text. Results are expressed as means ± SE.

† Statistically different ($P < 0.01$) from the control.

suggest that VCR at a concentration of 1 μM does significantly increase intracellular MTX. Since a 0.15-mg/kg VCR dose reaches a peritoneal fluid concentration of 1.4 μM at 1 minute after injection declining with a $T_{\frac{1}{2}}$ of 33.5 minutes thereafter, augmentation of uptake might have been expected with the concurrent administration of both agents. However, the rapid decline of VCR concentrations in vivo, in contrast to its constancy under in vitro conditions, may explain the observed difference. Furthermore, metabolism of VCR in vivo may diminish the concentration of the active moiety. At a VCR dose of 1.5 mg/kg, peritoneal fluid concentrations of 16.5 μM were observed 1 minute after injection declining to 7.3, 3.3, and 0.61 μM, respectively, by 30, 60, and 120 minutes. Thus, an augmentation of MTX uptake might have been expected at the 0-, 30-, and 60-minute intervals based on our in vitro studies. In contrast, VCR-induced augmentation of MTX uptake was only observed at the 0 time interval for all MTX doses and at the 30-minute time interval for the 350-mg/kg MTX dose. The reasons for the disparity between the in vivo and in vitro data are unclear. Studies performed to date in animals and man suggest minimal in vivo metabolism of VCR (14,20). However, since the pharmacokinetics of VCR in man suggest rapid clearance from the blood ($T_{\frac{1}{2}}\alpha$ = 0.74 minute and $T_{\frac{1}{2}}\beta$ = 7.4 minutes) with peak blood levels of 0.36 μM following an iv injection of 2 mg, in vivo augmentation of MTX uptake in human tumors in situ appears unlikely (14). If a VCR effect is to be achieved, it may be theoretically abetted by administering VCR 30 minutes after MTX administration. However, since a maximal MTX effect on tumor cells and normal tissues appears to require exchangeable intracellular MTX (9), the role of VCR in augmenting an already appreciable $[MTX]_i$ achieved by "high-dose" MTX might be questioned (*see* table 3). Logically, it might be argued that lower doses of MTX could be used if VCR-induced augmentation of MTX uptake could be demonstrated in a given tumor. However, the results of this study would suggest that even when VCR can be demonstrated to augment MTX uptake, an enhanced antitumor effect does not necessarily result. Only through clinical testing of a particular tumor can this question be resolved.

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Adriamycin and Mitomycin C: Possible Synergistic Cardiotoxicity [1]

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SUMMARY

Ninety-one patients with advanced breast cancer failing adriamycin (ADR)-containing combination chemotherapy were treated with a combination of mitomycin C (MMC) and megestrol acetate. Congestive heart failure (CHF) occurred in 14 (15.3%) of 91 MMC-treated patients compared to three (3.4%) of 89 patients treated with similar ADR-containing combination chemotherapy without MMC ($P = 0.01$). The median time from the last dose of ADR to evidence of CHF was 8.5 months for the MMC group compared to 1.5 months for the other group. A significantly higher incidence of late onset of CHF implicates MMC as a possible cardiotoxic agent.

[Cancer Treat Rep 62:1005–1008, 1978]

Anthracycline-induced cardiac damage is a dose-related phenomenon, and a rather well-defined threshold in cumulative dose exists below which the incidence of cardiomyopathy is low (1). Certain factors are known to increase the risk of cardiomyopathy including mediastinal irradiation (2,3), drugs such as actinomycin D (4), mithramycin (4), cyclophosphamide (5), and DTIC (6), hypertensive heart disease, and valvular heart disease associated with cardiac hypertrophy (7). The congestive heart failure has usually been reported to occur soon after the last dose of anthracycline (7). We have observed a significantly increased incidence of late onset of congestive heart failure (CHF) in advanced breast cancer patients previously treated with adriamycin (ADR) who subsequently received mitomycin C (MMC) as a second-line therapy.

MATERIALS AND METHODS

Between October 1975 and September 1976, 91 patients with advanced breast cancer were treated with a second-line therapy consisting of a combination of 160 mg/day of megestrol acetate and 20 mg/m^2 of MMC iv every 4–6 weeks. All patients were initially treated with various ADR-containing regimens combined with cyclophosphamide, methotrexate, vincristine, 5-fluorouracil, or ftorafur. The maximum dose of ADR was limited to 450 mg/m^2. All patients were monitored by history and physical examination, chest roentgenograms, and electrocardiogram (EKG) for any evidence of cardiac failure during ADR combination chemotherapy. None of the patients had any clinical evidence of CHF at completion of ADR therapy or at initiation of second-line therapy with MMC and megestrol acetate. When alerted to the possibility of increased incidence of CHF in the MMC-treated group, the charts of all patients who received MMC were reviewed. To compare the incidence of CHF in this group, the charts of 89 consecutive patients seen between June 1973 and September 1975 who were treated with similar ADR combination chemotherapy, but did not receive MMC as a second-line treatment, were also reviewed. In this review, risk factors were determined for all patients in both groups. These factors included age, any history of intrinsic heart disease (hypertension, valvular heart disease, or arteriosclerotic heart disease), prior irradiation to the left or right chest wall, total dose of ADR for each patient, and survival from the start of the first dose of ADR. The chest roentgenograms of all patients having any evidence of CHF were reviewed to determine the cardiac size, pulmonary vascular

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TABLE 1.—Distribution of risk factors in all patients

Risk factors	ADR and MMC	ADR	*P* value*
Total No. of patients	91†	89†	
Age in yrs			
Median	53	52	0.3
Range	29–78	25–74	
No. of patients			
Race			
Black	9	3	0.23
Latin American	3	3	
White	79	83	
Intrinsic heart disease			
Hypertension	9	11	0.77
Arteriosclerotic heart disease	1	0	
Radiation therapy			
Left peripheral lymphatics‡	15 } 30	15 } 27	0.74
Comprehensive§	15 }	12 }	
Right peripheral lymphatics‡	10 } 22	19 } 26	
Comprehensive§	12 }	7 }	
None	39	36	
Prior total ADR dose in mg/m²			
Mean	413.6	413.4	> 0.5
Range	100–530¶	90–550¶	
Survival from 1st dose of ADR in mos			
Median	19	22	0.08
Range	4–45	3–48	

**P* values for age and survival were done by the Mann-Whitney U test; for ADR a Student's t test was used and all others were compared by the chi-square test.

†All patients received concomitant cyclophosphamide with ADR.

‡Peripheral lymphatics includes supraclavicular or internal mammary lymph nodes; dose: 4500–5000 rads.

§Comprehensive includes peripheral lymphatics and chest wall irradiation; dose: 4500–5000 rads.

¶2 patients in MMC group and 9 patients in ADR group received > 450-mg/m² dose limit.

changes, and extent of any concomitant metastatic disease in the lungs or pleura. In patients who had evidence of CHF, the total voltage of QRS complexes in all six standard leads was determined at the termination of ADR therapy and at the onset of clinical evidence of CHF. Because of our unawareness of the cardiotoxicity of MMC, none of the patients had any EKGs performed during MMC therapy.

RESULTS

The two groups are compared in table 1. In both groups, the distribution of major risk factors which include hypertensive cardiovascular disease, irradiation to precordial and mediastinal areas, and total dose of ADR was similar. All patients received cyclophosphamide concomitantly with ADR.

The characteristics of patients who developed CHF in both groups are presented in table 2. Fourteen patients (15.3%) in the MMC-treated group developed CHF compared to three patients (3.4%) in the other group. These differences are statistically significant ($P = 0.01$). The median age was 53 years in the MMC group which is similar to the control population. None of the patients in the MMC group who developed CHF had pre-existing heart disease. However, two patients had left-sided radiation and two others had radiation to the right internal mammary lymph nodes. None of the patients in the MMC group who developed CHF had had the ADR dose exceed the 450-mg/m² limit, however, two of three patients in the ADR group had doses > 500 mg/m². The third patient in the ADR group had hypertension and developed CHF at an ADR dose of 400 mg/m².

TABLE 2.—Characteristics of patients with CHF

Patient No.	Age (yrs)	Total ADR dose (mg/m²)	Total MMC dose prior to onset of CHF (mg/m²)	EKG voltage†	Interval from last dose of ADR to onset of CHF (mos)	Prior irradiation to chest wall or mediastinum	Metastatic disease on chest roentgenogram	Comments
				ADR and MMC group				
1*	46	450	80	38/22	13	Yes	No	5000 rads to right internal mammary nodes
2	65	350	40	—	10	No	Yes	Few bilateral nodular lesions
3	51	450	55	93/28	21	No	No	
4	30	450	60	40/29	2	No	Yes	Few nodular pulmonary lesions
5	57	250	76	—	8	No	No	
6	31	340	60	47/44	5	Yes	Yes	Left-sided effusion and pulmonary metastases (few), 2000 rads to chest wall
7	65	420	20	57/22	9	No	Yes	Small left pleural effusion
8	49	400	60	—	4	No	No	
9	49	450	55	36/13	22	No	Yes	Small right pleural effusion
10*	55	150	20	24/14	2	No	Yes	Right pleural effusion (moderate)
11	78	450	56	77/55	13	Yes	No	5000 rads to left chest wall
12	60	450	40	36/17	11	No	Yes	Single coin lesion, left lung
13*	61	450	20	18/8	2	No	No	
14	58	200	80	47/22	5	Yes	Yes	5000 rads to left internal mammary nodes
				ADR group				
1	54	400	—	45/29	1.5	No	No	
2	31	550	—	48/29	1.5	No	No	
3*	52	520	—	59/57	3	No	No	

*These patients had autopsy.
†EKG voltage at completion of ADR/voltage at onset of CHF.

The median time to onset of CHF from the last dose of ADR was significantly longer (8.5 months) for the MMC group compared to the ADR group (1.5 months). CHF was usually observed after a median of three cycles (median dose, 55 mg/m²) of MMC therapy. In all patients with CHF there was cardiac enlargement on the chest roentgenograms and in three patients the possibility of pericardial effusion was considered because of marked cardiomegaly, but was ruled out by an echocardiogram and cardiac scan. There were significant drops in EKG voltage in standard leads, and these drops were observed from the last EKGs done at the conclusion of ADR therapy. Seven patients in the MMC group initially responded to digitalis and diuretic therapy but of these, five subsequently died from CHF. In the other seven patients, there was no response to diuretic and digitalis therapy. In the ADR group, two of three patients died from progressive metastatic disease, while one patient died from CHF. At autopsy, there was myocardial degeneration with moderate interstitial fibrosis in all patients, and none of these patients had arteriosclerotic or valvular heart disease.

DISCUSSION

The incidence of ADR-induced cardiomyopathy varies depending on the total cumulative dose. At a total ADR dose of 450 mg/m² (alone or in combination with cyclophosphamide) the reported incidence of cardiomyopathy has been negligible (7). The observed incidence of CHF in our ADR- and MMC-treated patients was significantly higher than that expected with ADR combination chemotherapy without MMC. Certain risk factors which are known

to increase the incidence of cardiomyopathy, such as mediastinal radiation, cyclophosphamide therapy, and hypertension, are comparable in the two groups. None of the MMC group had extensive pulmonary metastases to account for this increased incidence of CHF. The only other drug that these patients received concomitantly with MMC, megestrol acetate, is not known to cause significant fluid retention, as occurs with estrogens or androgens (8).

The delayed cardiac toxicity of ADR has been of considerable concern. However, large clinical experience with various anthracyclines in the past several years has not encountered this problem. In fact, the median time to onset of CHF after termination of therapy either with ADR or with daunorubicin has been reported to be 34 and 63 days, respectively (7,9). A much longer latent period from last dose of ADR to onset of CHF observed in our MMC-treated patients strongly suggests that MMC possibly enhanced the subclinical cardiac damage already induced by prior ADR therapy. It could be that patients in the MMC group had delayed onset of cardiomyopathy because of longer survival, but the median survival of the two groups is very comparable. There have been no reports of MMC as a potential cardiotoxic agent, but in previous studies with MMC either the patients had had no prior exposure to anthracyclines or they had received these two agents simultaneously but at rather low doses, and the median survival of these patients may not have been long enough to observe this interaction (10).

It is possible that the higher incidence of CHF with the MMC-treated patients could be partly explained on the basis of the not uncommon occurrence of anemia secondary to MMC therapy. However, the second line of therapy given to the ADR-treated patients included equally myelosuppressive drugs such as the nitrosoureas or vinblastine. Based on these data, it is suggested that MMC, like other alkylating agents, is a potential cardiotoxic agent. We suggest that patients who have had prior exposure to anthracyclines be monitored closely for the possibility of CHF during treatment with MMC.

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Treatment of Mycosis Fungoides Lymphoma: Effectiveness of Infusions of Methotrexate Followed by Oral Citrovorum Factor[1,2]

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SUMMARY

Eleven patients with stage II or III mycosis fungoides lymphoma were treated with a regimen consisting of iv infusions of methotrexate (MTX) (60–240 mg/m^2) administered sequentially and oral citrovorum factor. All 11 patients experienced a good to excellent response. Complete remissions were induced in seven of the 11 patients. In most cases remissions were sustained with weekly administration of low-dose (25–50 mg) MTX. Adverse drug reactions, such as leukopenia, anemia, and mucositis, were minimal in degree. Some unique adverse reactions included skin ulcerations (three patients), a leukocytoclastic angiitis (one patient), and painful hands and feet during MTX infusions (two patients). Our experience with this regimen has led us to conclude that it is safe and very effective in the treatment of stages II and III of mycosis fungoides.

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Mycosis fungoides lymphoma was first described > 170 years ago, yet the search for an effective treatment regimen has generally been unrewarding. A wide variety of physical and chemical agents have been used to treat mycosis fungoides. Except in isolated instances, treatment has been mostly palliative and has been characterized by uniform lack of success, ie, no clearing of disease and no improved survival (1–5). Systemic administration of cytotoxic drugs has proven to be the least promising form of treatment. Low-dose methotrexate (MTX) has been used frequently in an effort to ameliorate the signs and symptoms of disease in some cases of mycosis fungoides. However, after variable time periods (most often short), the disease, in most patients, becomes refractory. Published reports of results using low-dose MTX are very limited (6,7).

With this knowledge in mind, we elected to test the efficacy of moderate, intermittent doses of MTX followed by the administration of citrovorum factor (CF) in a group of patients with mycosis fungoides lymphoma.

MATERIALS AND METHODS

Eleven patients with clinical and histologic evidence of mycosis fungoides were studied. Approximately one half of the patients were studied at the Oncology Service, Departments of Medicine and Pharmacology, Yale University School of Medicine, and the other half were studied at the Dermatology Service, Roger Williams General Hospital, Brown University. Nine patients had received some form of previous therapy, most frequently topical mechlorethamine, electron beam, and multiple systemic agents. Two patients had not been treated previously (table 1).

Each patient was admitted either to the Clinical Research Center, Yale-New Haven Hospital, or the Clinical Investigation Unit, Roger Williams General Hospital. Prior to treatment, all patients were examined thoroughly for signs of systemic disease. Pretreatment examinations included a complete his-

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TABLE 1.—Patient characteristics

Patient No.	Age/sex	Disease stage*	Extracutaneous involvement	Previous therapy	No. of courses of MTX and CF	Disease response	
						Degree†	Duration (mos)
1	64/F	II	None	Prednisone, cyclophosphamide, azaribine, topical mechlorethamine, electron beam	5 iv, 4 oral	4+	24‡
2	41/F	III	Lymph nodes (dermatopathic); hepatomegaly (negative biopsy)	Azaribine, low-dose MTX	2 iv, 3 oral	3+	6
3	27/F	III	Lymph nodes (dermatopathic)	None	Weekly × 6 mos and biweekly × 1 yr, oral	4+	18
4	58/F	III	Lymph nodes (dermatopathic)	Electron beam, azaribine, bleomycin, prednisone, topical mechlorethamine	6 iv, 18 oral	4+	20
					2 iv, 9 oral§	4+	6
					3 iv, 10 oral	4+	18
5	78/M	III	Lymph nodes (sinus histiocytosis)	Radiation, local injections of mechlorethamine	5 iv	4+	6‡
6	59/M	III	Lymph nodes (abundant Sezary-type cells on light and electron microscopy); liver (mild fatty changes, periportal lymphocytosis)	Topical mechlorethamine	4 iv, 7 oral	4+	15
7	74/M	III	Lymph nodes (dermatopathic)	Topical mechlorethamine	2 iv, 2 oral	3+	78
8	60/F	II	Lymph nodes (sinus histiocytosis)	Azaribine, topical mechlorethamine, topical corticosteroids	4 iv, 17 oral	4+	40
9	65/F	II	None	Topical mechlorethamine	3 iv	4+	36
10	63/M	II	Lymph nodes (histiocytosis); hepatomegaly (mild fatty metamorphosis and nuclear pleomorphism)	None	2 iv, 4 oral	4+	24
11	50/M	II	Lymph nodes (dermatopathic)	Azaribine, topical mechlorethamine	5 iv, 6 oral, 5 iv¶	4+	26

*See fig 1.
†4+ (excellent) = 80%–100% clearing; 3+ (good) = 60%–79% clearing; 2+ (fair) = 40%–59% clearing; poor = 0–39% clearing.
‡Lost to followup.
§3 series of treatments.
¶ 2nd series of treatments.

tory and physical examination and a battery of laboratory studies which included a complete hemogram, SMA-12, bone marrow aspiration and biopsy, liver and spleen scan, multiple skin biopsies, lymph node biopsy (preferably lymph nodes draining active disease sites), percutaneous liver biopsy, chest roentgenogram, bone survey, and in two instances, lymphangiography (lymphangiography was performed only in the absence of palpable lymphadenopathy). After the initial diagnostic workup was completed, each patient's disease was staged according to criteria outlined in figure 1.

Using our method of disease classification (which is a prognostic classification as well as a guide to therapy), we have divided our stage I and II patients into two categories. Those patients without evidence of internal organ disease involvement are placed in stages IA and IIA. Those patients with positive evidence of internal organ involvement are placed in stages IB and IIB. Several studies have shown that the presence or absence of palpable lymph nodes and organomegaly in patients with mycosis fungoides lymphoma correlates well with prognosis (5,8). Therefore, in our system of classification, patients with any of the following clinical and/or laboratory abnormalities are considered to have internal organ disease: palpably enlarged lymph nodes, livers, and spleens, positive lymphan-

Stage I Macular erythematous and eczematous cutaneous lesions
A. Without internal organ disease
B. With internal organ disease*

Stage II Plaque and nodular cutaneous lesions
A. Without internal organ disease
B. With internal organ disease*

Stage III Tumors and ulcerations of the skin and Sezary-type disease

*In our staging system, patients with palpable lymph nodes, hepatosplenomegaly, and/or abnormal lymphangiograms as well as laboratory evidence of other organ system involvement are considered to have internal organ disease. All patients with or without evidence of lymphadenopathy and/or hepatosplenomegaly were subjected to lymph node and liver biopsy. See text for further explanations.

FIGURE 1.—Stages of mycosis fungoides lymphoma.

giography, dermatopathic lymphadenopathy, and/or specific mycosis fungoides infiltrates in various organ systems. Patients with Sezary-type mycosis fungoides are classified as having stage III disease.

MTX was administered in a 24-hour iv infusion of 5% dextrose and water at a dose of 1–5 mg/kg or 60–240 mg/m². Most patients were initially given the lower dose which was increased incrementally to larger doses with each subsequent treatment course. Eight hours after the end of the infusion, each patient was given 25 mg of CF orally. This dose of CF was repeated every 6 hours for a total of four doses. In each patient this regimen (or "course" of treatment) was repeated at least two additional times at 5-day intervals. (As experience was gained in the use of this form of therapy, the number of "courses" given to each patient was increased so that patients in the latter part of the study received an average of five courses of iv MTX and oral CF [table 1].) Patients who tolerated iv MTX and CF but whose disease had not completely cleared after three to five courses of treatment were changed to a regimen of oral MTX and CF. These patients were then followed as outpatients on a weekly basis until maximum clearing was achieved. (MTX [60–240 mg/m²] was divided into three equal doses and given every 8 hours; CF [25 mg] was given orally 6 hours after the last dose of MTX and continued for three additional doses as above.)

Prior to the administration of each course of MTX and CF, a cbc count and urinalysis were obtained. A complete SMA-12 chemistry profile was performed weekly. At the completion of each series of treatments, repeat skin biopsies were performed in all of our patients. Lymph node and liver biopsies were not repeated in our patients unless an increase in size was noted. Clinical assessment of the size of each patient's lymph nodes and liver was made on each examination. However, patient response was expressed as percent improvement of the skin as judged by the disappearance of or reduction in size of plaques, nodules, or tumors, and by the degree of healing of skin ulcerations. An excellent response was 80%–100% clearing of the skin, a good response was 60%–79% clearing, a fair response was 40%–59% clearing, and a poor response was any lesser degree of clearing.

Maintenance therapy in most patients consisted of low-dose oral MTX (25–50 mg) weekly (eight patients) or moderate-dose oral MTX (60–120 mg/m²) and CF (25 mg orally × four doses) weekly (three patients: 8 weeks, 17 weeks, and 18 months). Two patients who experienced disease exacerbations while receiving low-dose MTX were re-treated with a regular course of moderate-dose MTX and CF. One patient has been re-treated twice. Four patients supplemented low-dose oral MTX with frequent (usually daily) total body applications of mechlorethamine solution.

RESULTS

Table 2 summarizes the response of 11 patients treated with MTX and CF. All 11 patients had a measurable response to treatment with nine of the 11 patients having at least 80% clearing. The two patients who were re-treated both experienced ≥80% clearing after each treatment course. Complete remissions (CRs) were induced in seven of the nine patients who had an excellent response to treatment. Repeat skin biopsies in all of these patients were found to be negative. Lymph node regression was very pronounced in those patients with enlarged nodes prior to therapy. Remissions lasting from 6 to 30 months were maintained with weekly low-dose MTX. One patient with total body erythroderma was included in the group of patients experiencing CRs. Prior to treatment, this patient had experienced intractable pruritis. On physical examination the skin of his body was edematous and erythematous, the skin of his palms and soles was thickened and hyperkeratotic, and he had massively enlarged cervical, axillary, and inguinal lymph nodes.

His peripheral blood laboratory values were as follows: (*a*) upon admission: hemoglobin, 10.4 g/100 ml; hematocrit, 33%; wbc count, 12,500/mm³; polymorphonuclear neutrophils, 57%; lymphocytes, 26%; monocytes, 10%; eosinophils, 7%; and platelet count, 270,000/mm³; (*b*) interim values—during hospitalization: hemoglobin, 10.3 g/100 ml; hematocrit, 33%; wbc count, 14,100/mm³; polymorphonuclear neutrophils, 73%; lymphocytes, 13%; monocytes, 8%; and eosinophils, 6%; (*c*) after hospitalization: hemoglobin, 11 g/100 ml; hematocrit, 34%; wbc count, 19,000/mm³; polymorphonuclear neutrophils, 86%; bands, 1%; lymphocytes, 6%; monocytes, 4%; eosinophils, 2%; basophils, 1%; and platelet count, 320,000/mm³

TABLE 2.—Summary of patient response

Degree of response*	Disease stage II Skin	II Skin and internal	III Skin	III Skin and internal	Total responders
Excellent	2	3	—	4	9
Good	—	—	—	2	2
Fair	—	—	—	—	—
Poor	—	—	—	—	—

*See table 1 for definitions.

(5%–10% of the peripheral wbcs had the morphologic characteristics of Sezary cells). Sezary-type cells were also observed in the lymph node biopsies. Approximately 15 months ago, 20 months after treatment with MTX and CF was started, all systemic and topical therapy was stopped in this patient. To date he has shown no signs of disease recurrence. Two additional patients whose disease had been classified as stage III also experienced CRs. Although two of our patients with stage III disease did not experience an excellent response to therapy, we were able to ameliorate signs and symptoms of disease in one of the two patients for > 78 months.

Table 3 summarizes the number and kinds of adverse reactions experienced by the 11 patients. As would be expected, most reactions were noted in the hematopoietic system and the gastrointestinal tract. The severity of these reactions was surprisingly mild but a number of reactions proved to be of great concern to the patients who experienced them. Three patients developed ulcerations in the skin that was involved with disease. In a 47-year-old woman with stage III disease, approximately 80% of the skin ulcerated after two courses of treatment. Intensive topical care resulted in complete healing of the ulcerated sites. Six months later, the patient was able to tolerate a similar course of treatment without again experiencing this complication. In this patient, skin ulcerations occurred in sites that were not obviously involved with lymphoma as well as in sites that contained visible evidence of disease. Three patients experienced exacerbations of their cutaneous lesions during the first two to three courses of MTX. These exacerbations were characterized by increased edema and erythema at the involved sites as well as increased pruritis, burning, and ulceration. Three other patients complained of severe pain and burning in the palms and soles during each of their early infusions. Only one patient had evidence of disease involvement of the palms and soles. One of our patients developed palpable purpuric lesions of the lower extremities after the fifth course of treatment. Histologic examination of a lesion showed a leukocytoclastic angiitis. A subsequent course of treatment resulted in an outbreak of new palpable purpuric lesions on the lower extremities.

Bleeding and infection did not occur in any of the 11 patients. Measurable renal, liver, and pulmonary toxic effects also did not occur during the course of this study.

TABLE 3.—Adverse reactions

Type	No. of patients
Hematopoietic	
Wbcs—	
< 2000/mm³	0
2000–4000/mm³	7*
Hematocrit < 35%	5†
Platelets—	
< 100,000/mm³	0
100,000–200,000/mm³	6
Gastrointestinal	
Mucositis	
Mild	2
Severe	2
Nausea	3
Diarrhea	1
Skin	
Ulceration	
Mild	1
Severe	2
Vasculitis	1
Exacerbation of lesions	3
Other: pain in hands and feet	3

* Low wbc count in all isolated instances.
† Low hematocrit reading in all isolated instances.

DISCUSSION

Earlier reports by Hryniuk et al (9), Lefkowitz et al (10), and Mitchell et al (11) have demonstrated the effectiveness and relative safety of intermittent infusions of MTX followed by the administration of CF in the treatment of patients with refractory forms of acute leukemia and epidermoid carcinoma. More recent studies by Djerassi (12), Jaffe et al (13), and others have demonstrated the efficacy of much larger doses of MTX and "CF rescue" in the treatment of osteogenic sarcoma. However, the incidence of drug toxicity in these studies has been high and, in some cases, of a very severe degree.

Prior to these reports, Goldin et al had shown that parenteral CF given at an appropriate time interval (about 12–14 hours) after a large dose of a folic acid antagonist could obviate to a great extent host toxicity without impairing the antineoplastic effect of the antagonist (14). Sandberg and Goldin reported similar observations using oral CF (15). Skipper et al had previously postulated and shown that in cancer chemotherapy "cancer kill" is directly related to the dose of drug administered and the timing and/or frequency of drug administration (16). Ideally, a drug should be administered intermittently and during the period of peak DNA synthesis. Bruce et al further demonstrated that cytotoxic agents could be administered continuously for periods up to 24 hours, and while killing 99.9% of tumor cells, at least 20% of bone marrow stem cells would be spared (17).

This series of studies prepared the groundwork for additional examinations of the value of large doses of properly timed cytotoxic agents in the treatment of human cancer. Our interest in developing an effective regimen for the treatment of mycosis fungoides lymphoma and our experience using moderately large doses of MTX and CF in carcinoma of the head and neck stimulated us to try this form of therapy in patients with stages II and III mycosis fungoides. The rationale for the dosage and timing of MTX and CF used in this study is discussed in detail in the report of Mitchell et al (11).

In spite of the poor past experiences using systemic chemotherapeutic agents in the treatment of mycosis fungoides lymphoma, we continue to be optimistic in our approach to the treatment of this disease. Instead of the classic approach to treatment, ie, palliation, we are constantly seeking single and combination drug regimens that will give us the ability to offer patients with mycosis fungoides lymphoma the same dramatic improvement in prognostic outlook that has been experienced by patients with Hodgkin's disease, childhood leukemias, etc.

Using our present approach, once a diagnosis of mycosis fungoides has been established, each patient's disease is staged and a therapeutic regimen appropriate for that stage is outlined. Disease staging is dependent on cutaneous as well as systemic involvement.

Our results using moderate-dose MTX and CF in stages II and III of mycosis fungoides have been most encouraging. In fact, our enthusiasm for this regimen, based on the experience described in this study, is such that we feel it is the most effective single cytotoxic drug regimen for the treatment of mycosis fungoides. To our knowledge, the proportion of patients who experienced an excellent improvement of their disease exceeds that reported in other similar studies using cytotoxic agents, particularly MTX (5–7,18,19). Nine of our 11 patients achieved at least 80% clearing; seven of the 11 achieved CR of their disease. The duration of remission, though variable in each patient, is also very impressive.

Using weekly doses of MTX, usually 50 mg im, Haynes et al reported six CRs in a population of 30 patients (7). These remissions lasted from 5 to 20 months. Wright reported objective improvement in nine of 16 patients treated with 2.5–10 mg of oral MTX daily (6). There were no CRs induced with this regimen; however, a significant number of patients achieved 50%–90% clearing. Improvement in this group of patients lasted from 1½ to 24½ months. Epstein et al make reference to 82% of 45 patients improving after treatment with MTX, although drug doses and degrees of improvement were not described (5). Epstein's review of 144 patients with mycosis fungoides showed that 50% of those having late-stage disease, tumors, ulcers, or palpable nodes on examination were dead within 2½ years. The median survival in 21 patients with lymph nodes positive for malignancy at initial examination was 18 months (5). From among our group of 11 patients in whom followup in most cases has been > 36 months (seven patients, 40–120 months), we have experienced only one death. This patient, a 67-year-old woman in whom several courses of MTX and CF were used over a period of 10 years, died recently of a massive pulmonary embolus 3 months after high-dose MTX (500 mg/m^2) and CF had been successfully used.

It is our impression that the type of drug-related adverse effects experienced were exceedingly minor and were lesser in number and extent than those we have observed using any other single agent. Thus, our experience using moderate-dose intermittent infusions of MTX followed by CF has led us to conclude that this is a safe and very effective regimen for the treatment of mycosis fungoides lymphoma.

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Clinical Trial of Peptichemio in Solid Tumors of Childhood[1,2]

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SUMMARY

Peptichemio (PTC) was utilized in various advanced and disseminated tumors in children. With the schedule that was used, toxicity was mild. Immediate side effects included anaphylactoid reactions which required close supervision of the patient during infusion of the drug. PTC proved efficacious in advanced neuroblastoma. In patients with bone metastases, PTC has a prompt analgesic effect which seems to occur independently of any antitumor action.

[Cancer Treat Rep 62:1015–1019, 1978]

Peptichemio (PTC) is a mixture of six synthetic oligopeptides each of which contains an *m*-[di(2–chloroethyl)amino]-L-phenylalanine residue. Its antitumor activity, which has been ascribed to its alkylating group and to the antimetabolic properties of the peptide moiety (1), has been demonstrated in several animal tumor test systems, ie, Yoshida ascites sarcoma, Sarcoma 180 (1), and L1210 leukemia (2). In these studies, the therapeutic index of PTC compared favorably with that of melphalan. In a recent work however, the therapeutic index of PTC was not greater than that of melphalan and other alkylating agents when tested on several transplanted tumors of mice (3). PTC inhibits DNA- and RNA-dependent DNA polymerase, DNA synthesis, and protein synthesis in various mammalian cells in vitro and in vivo (1).

Compared to that of melphalan, the inhibitory effect of PTC on transformation of human lymphocytes in the presence of lectins in vitro is significantly smaller (2,4). Moreover, PTC has a selective effect on B cells where activation by mitogens is much more depressed by the drug than that of T cells by concanavalin A (ConA) or PHA (1,2). T cells obtained from patients given therapeutic doses of PTC remain reactive to ConA (5) while delayed-hypersensitivity skin reactions are inhibited but not suppressed in these patients (6). On the whole, PTC seems to partially spare T-cell functions.

The drug has already been clinically studied in adult tumors and found to be active in Hodgkin's and non-Hodgkin's lymphomas (2,7), leukemias (2,7,8), and lung (9,10), gynecologic (11), and bladder carcinomas (12).

It has not been extensively studied in pediatric tumors. Objective responses have been reported in ten children with various solid tumors resistant to conventional chemotherapy (13). Another study has shown a high response rate in neuroblastoma patients (14). The purpose of this study was to evaluate the efficacy and the toxicity of PTC in a series of children with various advanced or disseminated solid tumors. Some of the patients had already received other alkylating agents so that possible cross-resistance with PTC could be checked.

PATIENTS AND METHODS

Twenty-five children aged 8 months to 15 years with various generalized or advanced cancers were entered in this study between March 1974 and June 1977. Sixteen patients were given PTC for relapse of the primary tumor or metastatic disease resistant to other chemotherapy. In eight patients with inoperable or metastatic neuroblastoma, PTC was used as primary treatment. One patient with stage IV rhabdomyosarcoma, at first misdiagnosed as neuroblastoma, also received PTC as a first treatment. Patients were evaluated before PTC therapy with regard to tumor measurements as well as metabolic

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[2] Peptichemio: a mixture of 6 peptides of *m*-[di(2-chloroethyl)amino]-L-phenylalanine. Supplied by Instituto Sieroterapico Milanese, Milan, Italy, through the European Organization for Research on Treatment of Cancer (EORTC) Screening Group.

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TABLE 1.—Summary of responses for patients with neuroblastoma

Patient No.	Age (yrs)	Sex	Prior chemo-therapy	No. of courses	Mean dose (mg/kg/ course)	Disease stage	Results*	Duration (mos) of CR with PTC alone
1	4	M	Yes	41	5, 1st 2 yrs 3, last yr	IV (bone metastases)	E4 S2	42+
2	2	M	Yes	3	6	IV (bone metastases)	E1 S1	
3	5	F	Yes	8	6	IV (bone metastases)	E4 S2	3
4	2 8/12	M	Yes	18	6	III (increased VMA)	E4 S2	22+
5	4	M	No	8	6	IV (bone marrow)	E4 S2	6
6	9/12	M	No	3	6	IV	E3 S2	
7	9/12	F	No	2	5	IV	E3 S1	
8	6	M	No	2	5	IV	E2 S1	
9	10/12	M	No	3	6	III	E3 S2	
10	8/12	M	No	2	6	IV	E3 S2	
11	2 6/12	M	No	2	6	IV	E2 S1	
12	5	M	No	2	6	III	E2 S1	
13	2 3/12	F	No	2	6	III	E1 S0	

*Mode of evaluation of response according to the EORTC screening group: E1 = growth stopped or small decrease (< 20%); E2 = > 20% regression; E3 = > 50% regression; E4 = CR. S0 = status quo; S1 = evident improvement; S2 = disappearance of all symptoms.

and hematologic parameters. PTC therapy was started only if the wbc count was > 4000/mm^3 and the platelet count was > 100,000/mm^3, except for patients whose marrow aplasia was caused by neoplastic invasion (two patients with neuroblastoma) and who were given the drug despite having leukopenia at the start. For patients with neuroblastoma, complete remission (CR) was defined as not only disappearance of all clinical signs related to the presence of tumor but also normal values for dopamine, vanillylmandelic acid (VMA), and homovanillic acid.

PTC was available in ampules containing 10 or 40 mg of active product. It was diluted in 100 ml of 5% glucose in water, protected from light exposure, and administered as a 30–45-minute iv drip at a dose of 1.0–1.5 mg/kg daily for 3–5 consecutive days. Courses were repeated every 2–4 weeks according to drug tolerance.

Clinical response was evaluated according to the protocol of the EORTC clinical screening group (table 1).

RESULTS

A total of 595 PTC injections were given. Urticaria occurred during infusion once in three patients and systematically at each injection in two other patients. Three patients each developed a transient shock once during administration of the drug but tolerated further PTC therapy without complication. These shocks occurred within a 3-month period from June through August 1975, with samples from the same batch. Whether this complication was caused by impurities or alteration of some of the peptide components in that particular batch is not known.

In contrast with what has been described in adults, no gastrointestinal side effects were observed and antiemetics were not used. In some patients, not included in this report, vomiting did however occur when higher doses (2–3 mg/kg/dose) were given.

Hematologic toxic effects were moderate. In five children with neuroblastoma whose bone marrow was normal or scarcely invaded and who received

PTC as their first treatment, hematologic toxic effects became evident only after two courses of PTC given 10 days apart: leukopenia (< 3000 cells/mm³) occurred in three patients 4–7 days after the last injection and recovery was seen on the tenth day after completion of treatment. The wbc count nadir was from 1200 to 2700/mm³ in these three patients. Thrombocytopenia (< 100,000 platelets/mm³) was observed at the same time in one of these three patients and was present in another.

In patients receiving PTC after failure with conventional chemotherapy, hematologic tolerance was very variable.

In eight of 15 patients, leukopenia (< 3000 cells/mm³) developed within 2 weeks after the last injection of the first course and recovery (> 4000 cells/mm³) was observed on the 21st day after completion of the course. Thrombocytopenia (< 100,000 platelets/mm³) of 3–4 days' duration was observed. Two patients whose bone marrow was massively infiltrated by neuroblastoma cells and who had marked peripheral cytopenia received PTC as a first treatment and responded with a progressive improvement of peripheral blood parameters. There was no leukopenia-related infection or thrombocytopenia-related hemorrhage. One patient has been receiving PTC for 3 years on a monthly basis. Although his platelet count constantly oscillated between 20,000 and 80,000/mm³, he tolerated repeated courses without complication. Alopecia is almost constant after the third course.

Vein thrombosis often develops at the sites where repeated PTC infusions have been given. This side effect may create practical difficulties when using iv therapy.

RESPONSE

Objective and subjective response data for neuroblastoma patients are summarized in table 1. The staging system is that described by Evans et al (15). Patients 1–4 were given PTC for neuroblastoma resistant to conventional chemotherapy. Three of these patients entered CR.

Patient 1 presented with a tumor of the left adrenal, sparse bone marrow invasion, and bone metastases. He entered CR after complete excision of the primary tumor and weekly cyclophosphamide (CPM) plus vincristine (VCR). After a first relapse which occurred 14 months later, a second CR was induced by adriamycin (ADM). After 7 months of repeated ADM courses, a second relapse became evident: neuroblastoma cells were present in the bone marrow and multiple bone lesions were visible on a roentgenogram. Under PTC treatment, marrow infiltration was cleared within 3 weeks and increased VMA excretion returned to normal levels. The child is now well and has been without evidence of disease for 42 months since his last relapse.

Patient 2 had surgical excision of a stage II retroperitoneal neuroblastoma. She was given adjuvant chemotherapy (CPM + VCR + ADM) for 8 months and then developed bone metastases concurrently with increased VMA excretion. PTC induced a CR of 3 months' duration, after which metastatic disease appeared again.

Patient 4 had a stage III retroperitoneal neuroblastoma treated by subtotal excision, radiotherapy, and chemotherapy (CPM + VCR + ADM). Eight months after surgery, VMA excretion, which had returned to normal levels, was repeatedly found to be increased (60–84 μg of VMA/mg of serum creatinine). No other sign of relapse was discovered, except for a doubtful bone lesion. After two courses of PTC, VMA excretion returned to normal levels. The patient had maintenance treatment with PTC for 18 months and had no evidence of disease at 4 months after the end of the treatment.

Patient 5 had bone marrow invasion by neuroblastoma cells at the time of surgical removal of a retroperitoneal tumor. After one course of PTC, the bone marrow was free of tumor cells at three different sites. PTC was pursued until generalized relapse occurred 6 months later. Subsequently, the patient had only a transient response to multiple chemotherapy (VCR + ADM + CPM).

Patients 6–13 were given PTC as primary treatment for advanced neuroblastoma (stages III and IV). The main objective of this preoperative chemotherapy was to reduce the volume of the primary tumor in order to facilitate its surgical excision. In four of these patients there was a dramatic shrinkage of the tumor mass after two courses of PTC. Three patients had only a moderate decrease of the tumor extension (< 50%) but in two of these patients (Nos. 4 and 8) neuroblastoma cells disappeared from the bone marrow.

PTC was also given for a limited number of courses (one to four) to 12 patients with various tumors: three patients with Ewing's sarcoma, and the others with retinoblastoma, astrocytoma, medulloblastoma, osteosarcoma, hypernephroma, malignant teratoma, hepatoblastoma, T-cell lymphoma, and rhabdomyosarcoma. One objective response was observed in a child with stage IV rhabdomyosarcoma whose bone marrow was cleared of neoplastic cells after one course of PTC. This child had received no previous chemotherapy. The other patients were given PTC after failure of conventional drugs to control the disease. A partial regression (PR) of pulmonary metastases (< 50%) was observed in one patient with Ewing's sarcoma.

In two patients with Ewing's sarcoma who had multiple skeletal metastases, PTC had no evaluable objective effect. However, the drug had an evident analgesic action. Both patients who complained of excruciating pain experienced complete relief after the second PTC infusion. In these two patients, the elevated erythrocyte sedimentation rate (ESR) (> 100 mm/hour by the Westergren method) dropped to normal values within 1 week. The same subjective improvement was observed in a child with bone metastases from a retinoblastoma and in other patients with generalized disease.

DISCUSSION

The results reported here confirm those of De Bernardi et al (14) who observed that PTC induces PR or CR, albeit of short duration, in about 90% of patients with neuroblastoma. The results in patient 1 were particularly impressive in view of the advanced age of the patient and the multiple skeletal metastases, both very bad prognostic factors, the failure of conventional drugs to control the disease after they had been temporarily active, and the long remission following PTC treatment. Noteworthy is the efficacy of PTC in three patients whose tumor had become resistant to CPM, which suggests that cross-resistance does not necessarily exist between these two alkylating agents. The objective responses induced by PTC in neuroblastoma are in marked contrast with the inefficiency of melphalan in this same disease (16). At least for this particular tumor, the incorporation of the phenylalanine mustard moiety into small peptides has resulted in new specific antitumor properties. When used as primary treatment for neuroblastoma, PTC usually acts rapidly and without severe myelotoxic effects. It may therefore be particularly useful as a preoperative agent given to decrease the volume of bulky tumors.

PTC induced a PR in one patient with pulmonary metastases from a Ewing's tumor and in one patient who had bone marrow infiltration by rhabdomyosarcoma cells. The drug induced prompt pain relief in two patients with generalized Ewing's sarcoma who had severe pain caused by skeletal metastases. Since the eventual healing of bone lesions is difficult to evaluate objectively before a rather long period of time, we could not ascertain whether the rapid subjective improvement (concurrent with normalization of the ESR) was linked to an antitumor effect or implemented through some other mechanism of action. As the same subjective effect was observed in some patients with evaluable lesions but in whom no objective response was elicited, we favor the hypothesis that PTC has analgesic properties unrelated to its antitumor activity.

In patients receiving PTC in repeated courses or after previous intensive chemotherapy, myelotoxic effects were the main side effects. Sclerosis of the vein at the site of the infusion was frequently observed. In some patients, this benign side effect may make further iv therapy very difficult, if not impossible.

The results of this trial show definite activity of PTC in neuroblastoma. The drug should now be tried on a larger scale and be considered for use in multiple-drug protocols in this disease. Because of the phlebothrombotic effect of PTC, use of the drug should be restricted to the induction phase of treatment.

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Combination Chemotherapy for Ovarian Carcinoma With Cyclophosphamide, Adriamycin, and *cis*-Dichlorodiammineplatinum(II) After Failure of Initial Chemotherapy [1,2]

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SUMMARY

Twenty-four patients who had stage III or IV ovarian adenocarcinoma and had failed prior chemotherapy were treated with cyclophosphamide, adriamycin, and *cis*-dichlorodiammineplatinum(II) every 3 weeks. Twelve patients had objective responses and an additional six had subjective improvement. The median duration of survival for responders is 9 months. There were no instances of nephrotoxicity. Fluid management consisted of 1000–2000 ml of iv fluid without Lasix- or mannitol-induced diuresis. Patients responded after having failed radiotherapy or multiple-drug chemotherapy, including some who were 70–75 years of age or were > 50% bedridden. Patients with one- or two-drug prior chemotherapy had a higher response rate (ten responses among 17 patients) than those previously treated with three or more drugs (two responses among seven patients).

[Cancer Treat Rep 62:1021–1023, 1978]

This paper describes the results of treatment with a new drug combination, CAP (cyclophosphamide [CYC], adriamycin [ADM], and *cis*-dichlorodiammineplatinum(II) [DDP]), for advanced ovarian adenocarcinoma resistant to initial chemotherapy. CYC was added to a DDP-ADM combination (2). CYC is synergistic with both DDP and ADM in some animal systems (3,4), and has recognized action alone against ovarian cancer (5).

The efficacy of the individual drugs as initial or secondary treatment for ovarian cancer has not been precisely defined. Secondary therapy with CYC may produce response rates of approximately 40% in combination with 5-fluorouracil, but responses are of short duration (Bruckner H, unpublished data). Secondary therapy with ADM may produce response rates near 50% (6), but this is probably the exception. Response rates are usually lower, especially at a small dose of 30 mg/m^2. DDP produces response rates of 25% as secondary therapy (7,8).

The overall result reported in this paper of a 50% response rate with a 9-month median duration of survival after initial therapy has failed is better than the results of these earlier trials.

PATIENTS AND METHODS

From June 1976 to April 1977, all patients with advanced adenocarcinoma of the ovary were treated with CYC (300 mg/m^2), ADM (30 mg/m^2), and DDP (50 mg/m^2) as consecutive bolus injections once every 3 weeks. Patients were eligible for the study provided that they had: (*a*) failed prior chemotherapy; (*b*) histologically proven papillary serous, pseudomucinous, or solid adenocarcinoma; (*c*) proven measurable recurrence; (*d*) an expected survival of > 1 month; (*e*) a BUN and creatinine of < 25 and 2.2

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mg/100 ml respectively; and (*f*) wbc and platelet counts of > 4000 and 100,000/mm^3 respectively. Patients were ineligible if they had any of the following: (*a*) a performance status of 4 (completely bedridden); (*b*) a history of heart disease (recent infarction or arrhythmia); or (*c*) prior treatment with DDP.

Doses of ADM and CYC were reduced by 25% if the nadir wbc or platelet counts were < 1000 or < 50,000/mm^3 respectively and by 50% if the counts were < 500 or < 25,000/mm^3 respectively. Doses of ADM were reduced by 50% if the bilirubin was > 1.2 mg/100 ml or were withheld if it was > 2 mg/100 ml.

The serum BUN and creatinine were measured within 24 hours of starting treatment. Patients with a BUN and creatinine of < 25 and 2.2 mg/100 ml respectively were advised to drink 1000–2000 ml of fluids during the 24 hours preceding chemotherapy.

Immediately before treatment, patients were given another 1000–2000 ml of iv (5% dextrose, 25% saline) fluid. The patient was given chemotherapy only after urine production increased to the rate of 50–100 ml each hour. In this series, no patient required Lasix or mannitol to induce diuresis.

If the BUN or creatinine was abnormal before treatment, the patient was given 1000–2000 ml of iv fluids over 24 hours. If the BUN and creatinine returned to < 25 and 2.2 mg/100 ml respectively the patient was treated with DDP following infusion of an additional 1000–2000 ml of iv fluids.

Treatment with CAP was continued until progression of the tumor or side effects of chemotherapy prevented additional treatment. If the cumulative dose of ADM reached 400 mg/m^2, treatment was continued without ADM. The dosage of DDP was not adjusted or withheld except for nephrotoxicity or neurotoxicity. If the wbc count was < 4000/mm^3 21 days following treatment, treatment was continued with DDP alone until the wbc count recovered to 4000/mm^3.

Definitions of response were: *objective*—≥ 50% reduction in the product of two measurable tumor diameters or complete disappearance of ascites or pleural effusion in the absence of any intracavitary or diuretic treatment; *complete*—complete disappearance of all physical and radiologic evidence of tumor; *status quo*—disease stabilization, no new evidence of tumor or worsening of symptoms with a < 25% change in tumor size; *subjective*—performance status improved with no change in objective evidence of tumor; and *progression*—> 25% increase in the product of tumor diameters or new pathologic fluid or decrease in performance status. All responses were defined as those lasting ≥ 2 months. Definitions of performance status were: 1 = no symptoms; 2 = < 50% of time in bed due to tumor; 3 = > 50% of time in bed due to tumor; and 4 = > 75% of time in bed due to tumor. In the following text "responses" of the status quo and subjective type are described but are classified as objective failures of treatment.

RESULTS

Twelve of 24 patients achieved objective responses (table 1); two responses were complete. All patients with a poor performance status, advanced age, and prior radiotherapy had ≥ 50% response rates (table 1). Patients treated with only one or two drugs in the past had a higher frequency of response (ten responses among 17 patients) compared to patients treated with three or more drugs (two responses among seven patients). This trend is not statistically significant thus far. Both responders and nonresponders to prior radiotherapy or chemotherapy responded to treatment with CAP. The median duration of survival was 7 months for all patients and 9 months for responders. Four responders have survived > 1 year; no nonresponder survived 1 year.

Side effects of combination chemotherapy were well-tolerated, reversible with treatment, and predictable. Fifty percent of the patients required blood transfusions because of anemia. Twelve patients required a 25% dose reduction and six patients required a 50% dose reduction for leukopenia. None required dose modification for thrombocytopenia. (DDP alone in these doses can sometimes produce severe and prolonged thrombocytopenia in similar patients; Bruckner H, unpublished observation.)

TABLE 1.—Effect of prior treatment, age, and performance status on response rates to CAP in 24 patients with ovarian cancer

Characteristic	No. of responses		
	Objective	Subjective	Failure
Prior radiotherapy	4	2	1
No radiotherapy	8	4	5
Prior drugs			
1 or 2	10	5	2
3 or 4	2	1	4
Age (yrs)			
40–49	1	1	2
50–59	2	3	1
60–69	4	2	2
70–75	5	—	1
Performance status			
1	4	1	1
2	2	4	2
3	6	1	3
Total No. of patients	12	6	6

There were no instances of nephrotoxicity. Nausea and vomiting were regularly produced by treatment. There was one instance of self-limiting moderate hematuria that was possibly due to CYC.

DISCUSSION

CAP is a new regimen for ovarian carcinoma which produced 12 objective responses among 24 patients resistant to initial chemotherapy and polychemotherapy with CYC, ADM, 5-fluorouracil, and actinomycin D (two patients). Old age, prior radiotherapy, poor performance status, and resistance to initial chemotherapy or polychemotherapy did not preclude a response. The treatment produced no instances of unmanageable side effects.

Each of the three drugs has demonstrated activity against ovarian cancer as a single drug (2,5–11) and in combination (12,13). DDP was more active used in combination with ADM as initial chemotherapy compared to DDP alone or thioTEPA alone or in combination with methotrexate (2, 7). DDP alone was not compared to the ADM-DDP combination in a prospective controlled trial for patients with ovarian cancer failing prior chemotherapy. In an uncontrolled comparison, the ADM-DDP combination produced a better response rate (47%, seven responses among 15 patients)[5] than DDP alone (25%, three responses among 12 patients) for patients who had failed chemotherapy with only an alkylating agent with or without methotrexate (2,6,7). Similar patients treated with CAP had a further improvement in their response rate (59%, ten responses among 17 patients) compared to patients treated with ADM-DDP. These differences describe an apparent trend to improved response rates with DDP in combination therapy with additional active drugs. However, these were uncontrolled trials and the differences are not statistically significant.

These findings could support a recommendation for a controlled trial comparing CAP to ADM-DDP as initial therapy for ovarian adenocarcinoma. The merit of using a larger dose of CYC, ADM, or DDP in the CAP regimen during initial or maintenance therapy remains to be determined. These response rates with DDP used in combination chemotherapy are the highest reported to date for the treatment of ovarian cancer resistant to conventional therapy.

[5] Wallach R, Bruckner HW, Cohen CJ, et al. Combination chemotherapy of ovarian carcinoma with adriamycin and *cis*-diamminedichloroplatinum(II) after failure of initial chemotherapy. In preparation.

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Pharmacokinetic Considerations in Evaluating the Effects of Tetrahydrouridine on 5-Azacytidine Chemotherapy in L1210 Leukemic Mice [1,2]

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SUMMARY

The pharmacokinetics of 5-azacytidine (5-azaCR) and tetrahydrouridine (THU) were considered in evaluating the effect of THU on chemotherapy with 5-azaCR in L1210 leukemic mice. The administration of three different dose levels of THU and 5-azaCR ip in either a 6- or 72-hour infusion gave minimal increases in therapeutic effect. At the high-dose combinations (except in the 72-hour infusion), THU appeared to enhance toxicity. Toxicity, however, occurred only after exceeding a theoretic plasma concentration for 5-azaCR of 61 μg/ml. THU was effective in increasing the excretion of 5-azaCR by sixfold and in altering its urinary metabolites when given simultaneously with or up to 1 hour prior to 5-azaCR.

[Cancer Treat Rep 62:1025–1032, 1978]

5-Azacytidine (5-azaCR), a pyrimidine nucleoside analog of cytidine, was shown to be effective against murine L1210 leukemia in vitro (1,2) and in vivo (1), and against lymphoid leukemia in AK mice (3). Effective treatment of acute leukemia with 5-azaCR has been demonstrated (4–9). The compound inhibits DNA (1,10,11), RNA (11,12–14), and protein synthesis (15–17) and is incorporated into DNA and RNA (1,13). The nucleotide derivatives of 5-azaCR inhibit de novo pyrimidine biosynthesis by blocking orotic acid decarboxylase (18). Although some studies have shown that 5-azaCR is predominantly S phase specific (1,19,20), it was suggested that the compound could impair cell structures in resting cells (21). 5-AzaCR also has antibacterial properties (3,15,22).

5-AzaCR, when administered by rapid iv injection, produced hematologic toxic effects, nausea, vomiting, and diarrhea in most patients (6,7,9,23,24). The administration of 5-azaCR by constant infusion over a period of several days reduced the occurrence of gastrointestinal toxic reactions in patients (25–27) and led to improved chemotherapy against several human tumors (26).

Another nucleoside analog, tetrahydrouridine (THU), was shown to be a potent inhibitor of cytidine deaminase from bacterial (28) and human liver (29) and leukemic granulocytes (30). The compound was not metabolized and was rapidly excreted by various animal species (31), which presumably accounts for its lack of toxicity and antitumor activity (32). It was recently demonstrated that the antitumor activity of 5-azaCR was enhanced by THU when both drugs were administered orally to L1210 leukemic mice (33). Concentrations of 5-azaCR equivalents in blood after oral administration of 5-azaCR and THU were three times higher than after 5-azaCR administration alone by the same route. However, therapeutic enhancement of 5-azaCR by THU was not seen when both agents were administered ip. This difference in therapy between orally and ip administered drug may be associated with differences in their pharmacokinetics.

In this study, we have taken into account the pharmacokinetics of each agent in evaluating the

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[3] Arthur D. Little, Inc, Cambridge, Mass.

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potential of THU in optimizing 5-azaCR therapeutics.

MATERIALS AND METHODS

Female BDF_1 mice weighing approximately 20 g were used. Animals were inoculated ip on Day 0 with 1×10^5 L1210 leukemia cells and treatment was started on Day 1. Mean survival times and evaluation of data were determined according to protocols by Geran et al (34).

THU was obtained from the NCI (Bethesda, Md) and 5-azaCR was obtained from Monsanto Research Corp (Dayton, Ohio) through the NCI. The materials were prepared fresh daily, dissolved in sterile NaCl, 0.9%, and administered as described below.

In the infusion studies, loading doses in 0.5 ml were administered ip immediately prior to infusions. Infusion doses in 2.44 ml were administered over a 6-hour period from 5-ml plastic syringes and through Intramedic polyethylene tubing (internal diameter [ID], 0.011 inch, outside diameter, 0.024 inch, 52 cm in length) which was attached to 27.5-gauge needles. The free end of the tubing was cut diagonally and was inserted about 1 inch into either of the lateral tail veins. After insertion of the tubing, the tail was wrapped with a 3 × 7.5-cm strip of Elastoplast adhesive bandage to secure the tubing to the tail. The animals were placed in separate compartments in a common container and the tubing was suspended from above to allow the animal freedom of movement. The rate of infusion, 6.8 μl/minute, was controlled by a Harvard Infusion-Withdrawal Pump, model 961 (Harvard Apparatus Co, Inc, Dover, Mass). The experimental design accommodated the infusion of 12 mice/day.

In experiments where Alzet osmotic minipumps (Alza Corp, Palo Alto, Calif) (fig 1) were used, the animals were immobilized with sodium pentobarbital, 50 mg/kg ip, prior to implanting the minipump sc in the dorsal region just below the neck. Aseptic conditions were not strictly adhered to but the drug solutions were sterile and applied to the minipumps as described by the manufacturer. The minipumps remained in the animals for the duration of the experiments. The minipump has a capacity of 170 μl and consists of a collapsible reservoir surrounded by a sealed layer of osmotic driving agent which is the energy source. The energy source, contained by a rate-controlling semipermeable membrane, imbibes water from the aqueous environment of living tissue and generates hydrostatic pressure on the reservoir, which collapses and forces the contents of the reservoir through the delivery portal.

The in vitro rate of release of 4-^{14}C-5-azaCR was measured in duplicate as described below. The minipump was filled with the 11.9-μg (180 microcuries/mg) labeled compound and assembled according to the manufacturer's recommendations. The capsule was submerged in a stoppered glass vial containing 10 ml of saline and incubated at 37°C for 10 days. Aliquots of the saline were removed daily and counted for radioactivity and the averaged values were plotted against incubation time.

The manufacturer's quoted delivery rate of the pump was 2.5 μl/hour for 3 days, and, under our experimental conditions, the delivery rate was 1.34 μl/hour for 4.5 days. The observed rate accounted for 96% of the total radioactivity.

High-pressure liquid chromatography was carried out on a Waters Model ALC-202/R-401W/UV liquid chromatograph (Waters Associates, Inc, Milford, Mass). Five-microliter samples were analyzed on columns (15 cm × 4.2 mm ID) packed with Merck LiChrosorb Si-60 (5 μ) (EM Laboratories, Inc, Elmsford, NY). The effluent was chloroform:methanol:water:acetic acid (30:10:1.7:0.42) with a flow rate of 1 ml/minute at 2000 psi.

RESULTS

Chemotherapy of 5-AzaCR and THU Administered Ip

It has been demonstrated that THU markedly enhanced the therapeutic effect of cytosine arabinoside (ara-C) administered orally in the treatment of L1210 leukemic mice; however, THU had little effect on the activity of ara-C administered ip (32). Similar results were observed with THU and 5-azaCR (33). In the latter study, single and daily dose schedules were used with varied total doses of 5-azaCR and a constant total dose of THU.

The therapeutic effects of 5-azaCR and THU administered alone or in combination to L1210 leukemic mice are shown in table 1. The total dose of both drugs was varied and given every 3 hours over a 24-hour period. All of the dose combinations of 5-azaCR and THU were not significantly better in enhancing chemotherapy than the lowest dose of 5-azaCR alone. Although without toxicity and antitumor activity itself, THU was capable of enhancing the toxicity of 5-azaCR. This enhancement of toxicity by THU was also observed by Neil et al (33).

Effect of THU on the Urinary Excretion of 4-^{14}C-5-AzaCR

THU has been shown to be effective in increasing the plasma levels of ara-C and 5-azaCR administered orally to L1210 leukemic mice (32,33). In this study, we have evaluated the effect of THU on the

TABLE 1.—Therapy for L1210 leukemic mice (CDF_1) with combinations of 5-azaCR and THU*

THU (mg/kg)	%ILS of 5-azaCR at a dose (mg/kg) of—†					
	0	19.2	28.8	44	64	96
0	0	75	57	28	Toxic	
3.8	0	91	60	Toxic		
5.8	0	78	Toxic			
8.8	0	62	Toxic			
12.8	0	61	Toxic			
19.2	0	55	Toxic			

*Mice were inoculated on Day 0 with 1×10^5 L1210 leukemic cells and therapy was administered on Day 1 only.

†Values shown for 5-azaCR and THU are total doses given over a 24-hr period. One eighth of the total dose was given ip every 3 hrs over the 24-hr period. Both drugs were administered in molar ratios of 5:1 (5-azaCR:THU) and results are expressed as %ILS (increase in lifespan).

urinary excretion of 5-azaCR and its metabolites. Figure 2 demonstrates the influence of THU administered to L1210 leukemic mice with 4-^{14}C-5-azaCR at molar ratios of 0.01:1 to 2:1, respectively. These doses were effective in increasing the excretion of 5-azaCR and altering the profile of its urinary metabolites. High-performance liquid chromatographic analyses of urine showed that the levels of metabolites I, II, and V decreased, whereas the level of metabolite VI increased with increasing concentrations of THU. Metabolite III, however, was unaffected.

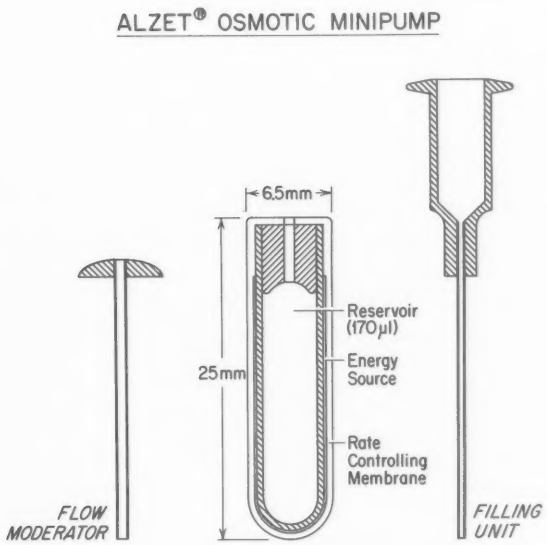


FIGURE 1.—Alzet osmotic minipump.

THU was also administered to mice at various times prior to 5-azaCR (table 2). The excretion of 5-azaCR and its metabolites was remarkably invariable, as judged from the small average deviation for the three control experiments. When THU was given simultaneously with 5-azaCR, there was a sixfold increase in the urinary excretion of the unchanged drug. The effect of THU on the profile of the urinary metabolites was similar to the data shown in figure 2. THU given 10 minutes or 1 hour prior to 5-azaCR had the same effect on the metabolite profile as the simultaneous administration of the compounds. THU, however, had no apparent effect on the excretion of 5-azaCR or its metabolites when THU was given 6 hours prior to 5-azaCR. This finding is not surprising since most of the THU administered to mice can be excreted unchanged within 6 hours (31).

6-Hour Infusion of 5-AzaCR and THU

Treatment of patients with 5-azaCR by rapid iv infusion (9) or intermittently over a period of several days (24) produces hematologic toxic effects, nausea, and vomiting. On the other hand, administration of the drug by constant infusion was shown to be clinically better (25–27) and resulted in higher concentrations of the drug in plasma (27). Since administration of the drug to patients by constant infusion was superior to other schedules, the effect of THU and 5-azaCR given by constant infusion was evaluated.

Models for the pharmacokinetics of 5-azaCR and THU after rapid iv injection were devised from our data[5] for 5-azaCR and those of Mellett et al (31) for

[5] Arthur D. Little, Inc. Interim Progress Report: Physiological Disposition and Mode of Action. Submitted to the DCT, NCI, Sept 1974, p 113.

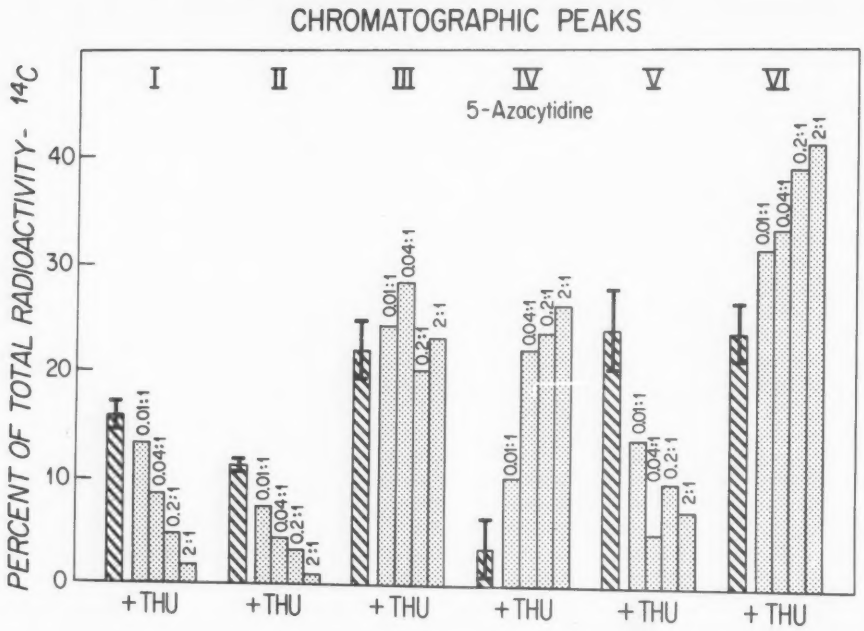
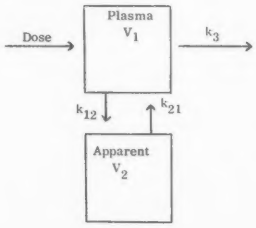


FIGURE 2.—Influence of THU on 5-azaCR urinary metabolites in BDF_1 mice. THU doses were administered at the designated molar ratios with respect to 5-azaCR and mice received 50 mg/kg of 5-azaCR. High-performance liquid chromatographic analyses were performed on cumulative 8-hr urine samples. Excretion recoveries during this collection interval ranged from 50% to 71%. The control values represent the means of 5 experiments ± SD.

THU. The calculated parameters are as follows: V_1, 50 ml/kg; V_2, 300 ml/kg; k_{12}, 50% min^{-1}; k_{21}, 0.33% min^{-1}; and k_3, 0.33% min^{-1} for 5-azaCR, and V_1, 50 ml/kg; V_2, 400 ml/kg; k_{12}, 50% min^{-1}; k_{21}, 1% min^{-1}; and k_3, 1% min^{-1} for THU.



As seen in figure 3A, if THU and 5-azaCR were given by rapid iv injection, THU would be present at effective molar ratios to 5-azaCR in plasma for < 2 hours. Furthermore, if both compounds were given by infusion, THU would reach its plateau approximately 3 hours after the start of a 6-hour infusion, whereas 5-azaCR would reach only 74% of the plateau level by 6 hours (fig 3B). Therefore, to maintain a constant molar ratio of both drugs, a loading dose of each drug is required prior to a 6-hour infusion (fig 3C and D). The doses and infusion rates used in subsequent studies are summarized in table 3.

The results from 6-hour infusions of 5-azaCR and THU to L1210 leukemic mice are shown in table 4. Survival times for doses of 5-azaCR of 25 and 50 mg/kg were not significantly different from identical doses which included THU at molar ratios of 5:1 (5-azaCR:THU). Doses of 5-azaCR > 50 mg/kg were toxic and THU appeared to enhance this toxicity. Identical doses of both drugs were also administered ip for comparison. The survival times from both routes of drug administration were similar (table 4).

72-Hour Infusion of 5-AzaCR and THU

Drug combinations were administered sc to mice for several days with the Alzet osmotic minipumps. The theoretic plasma concentration of 5-azaCR and THU is shown in figure 4. THU and 5-azaCR would reach 96% of their plateau concentration within 3 and 14 hours, respectively, and the plateau concentrations of 5-azaCR delivered by the minipumps were approximately one seventh of that delivered during a 6-hour infusion (table 3). Table 5 demon-

TABLE 2.—Effect of THU on the urinary metabolites of ^{14}C-5-azaCR in BDF$_1$ mice*

Drug and time†	Metabolite and drug‡ (5-azaCR) I	II	III	IV	V	VI	% of dose administered
5-AzaCR	16.4 ± 0.8	11.4 ± 0.4	20.3 ± 2.0	3.7 ± 2.5	25.7 ± 3.4	22.5 ± 1.5	50.1
5-AzaCR:THU, 0 min	4.8 ± 0.4	3.4 ± 0.6	20.0 ± 2.3	23.6 ± 3.7	9.5 ± 1.0	38.7 ± 2.3	53.3
5-AzaCR:THU, 10 mins	2.8	3.2	24.2	20.5	8.8	40.5	50.7
5-AzaCR:THU, 1 hr	3.1	4.6	20.2	17.7	10.9	43.5	51.3
5-AzaCR:THU, 6 hrs	12.6	11.5	20.2	10.4	19.0	26.2	60.4
5-AzaCR:THU, 24 hrs	13.0	13.9	17.3	9.9	21.5	24.3	56.7
						(mean ± SD)	53.8 ± 4

*THU (10 mg/kg) and 5-azaCR (50 mg/kg/3 microcuries-^{14}C) were administered at a molar ratio of 1:5.
†THU was administered prior to 5-azaCR.
‡% of recovered dose of each metabolite and drug. First 2 lines are means (± SD) of 3 experiments utilizing 3, 5, and 3 animals/experiment: ± values are average deviations. Remaining experiments utilized 3 animals/group.

TABLE 3.—Theoretic pharmacokinetic values for rapid injection and infusion schedules

Total dose (mg/kg)	Total C × t (mg/hr/kg)	Maximum plasma concentration* (μg/ml)	6-hr infusion Loading dose (mg/kg)	Infusion rate (mg/kg/hr)	Plasma plateau concentration (μg/ml)	72-hr infusion Infusion rate (mg/kg/hr)	Plasma plateau concentration (μg/ml)
			5-AzaCR				
25	320	71.5	10.7	2.4	30.6	—	—
50	640	143	21.4	4.8	61.2	0.74	9.47
100	1280	286	42.8	9.6	122.4	1.47	18.94
			THU				
5	9.6	11.1	0.63	0.73	1.4	—	—
10	19.2	22.2	1.26	1.45	2.8	0.14	0.29
20	38.4	44.4	2.52	2.9	5.6	0.28	0.58

*Based on rapid injection of total dose.

TABLE 4.—Therapy for L1210 leukemic mice with combinations of 5-azaCR and THU*

Dose of 5-azaCR† (mg/kg)	Ip infusion 5-AzaCR‡	%ILS	5-AzaCR + THU‡	%ILS	6-hr infusion 5-AzaCR‡	%ILS	5-AzaCR + THU‡	%ILS
0 (control)	10.7 ± 2.7				10.7 ± 2.7			
25	15.8 ± 3.2	48	15.7 ± 4.1	47	15.4 ± 3.3	44	17.4 ± 3.5	63
50	15.6 ± 2.2	46	16.5 ± 3.6	54	15.3 ± 2.4	43	14.3 ± 5.6	34
100	9.9 ± 5.7	Toxic	5.3 ± 4.5	Toxic	7.4 ± 6.7	Toxic	4.0 ± 2.7	Toxic

*Mice were inoculated on Day 0 with 1 × 10^5 L1210 leukemic cells and therapy was administered on Day 1. Controls received saline only.
†5-AzaCR and THU, where indicated, were administered in molar ratios of 5:1 (5-azaCR:THU) either by infusion after an initial ip loading dose or ip.
‡Values are mean survival time (days) ± SD.

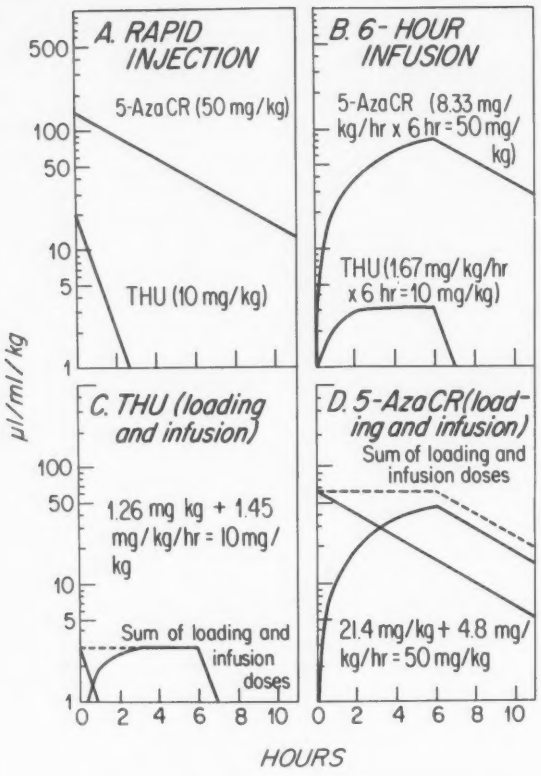

A. RAPID INJECTION
5-AzaCR (50 mg/kg)
THU (10 mg/kg)
B. 6-HOUR INFUSION
5-AzaCR (8.33 mg/kg/hr x 6 hr = 50 mg/kg)
THU (1.67 mg/kg/hr x 6 hr = 10 mg/kg)
C. THU (loading and infusion)
1.26 mg kg + 1.45 mg/kg/hr = 10 mg/kg
Sum of loading and infusion doses
D. 5-AzaCR (loading and infusion)
Sum of loading and infusion doses
21.4 mg/kg + 4.8 mg/kg/hr = 50 mg/kg
μl/ml/kg
HOURS

FIGURE 3.—Theoretic plasma concentrations of 5-azaCR and THU after rapid injection (A), after 6-hr infusion (B), and after a loading dose and infusion (C and D). The molar ratio of the theoretic administered doses of 5-azaCR and THU was 5:1 in each instance.

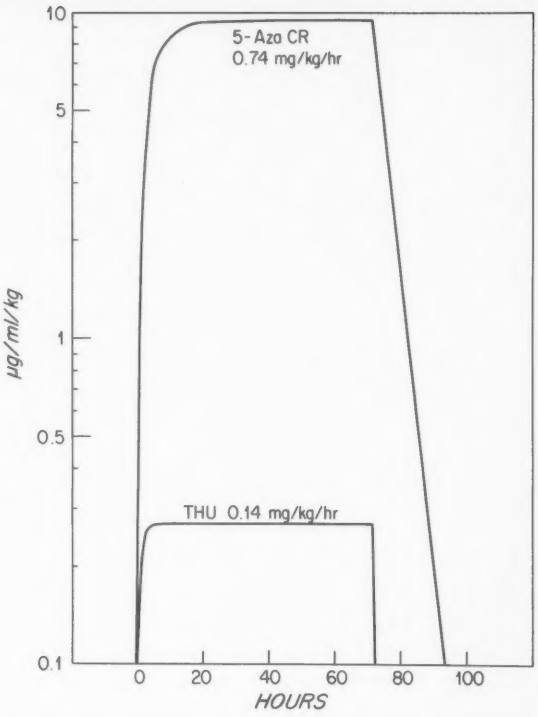


FIGURE 4.—Theoretic plasma concentrations of 5-azaCR and THU during 72-hr infusion. The total doses were: 50 mg/kg (5-azaCR) and 10 mg/kg (THU), and the molar ratios of the total doses were 5:1.

strates the results of studies from L1210 leukemic mice implanted with minipumps. The doses of 5-azaCR and THU tested were not effective in producing significant increases in survival times over doses of 5-azaCR alone. Survival times were similar to dose combinations given ip except at the highest dose. In this case, the 5-azaCR and 5-azaCR-THU doses given by the minipumps were not toxic as observed by the ip route and as noted earlier from the 6-hour infusion study.

DISCUSSION

Studies by Neil et al (33) showed that THU enhanced the chemotherapeutic effectiveness of 5-azaCR if both were given orally but not if given ip. One interpretation of these results suggests that differences may exist in the pharmacokinetics of each drug given by both routes. In the present study, we have considered the pharmacokinetics of both agents in evaluating the effect of THU or 5-azaCR chemotherapy in L1210 leukemic mice.

Therapeutic response to 5-azaCR and THU given ip indicated that THU minimally enhanced therapy and maximally enhanced toxicity (table 1). THU was shown to affect the urinary pattern of 5-azaCR equivalents. The pattern was dependent both on schedule (table 2) and dose (fig 2) of THU. After taking into account pharmacokinetic parameters for each compound, we compared the concentration × time (C×t) of 5-azaCR for three schedules, rapid injection, 6- and 72-hour infusion, and for several doses: 25, 50, and 100 mg/kg at constant 5-azaCR-THU molar ratios. Under these regimens, the maximum plasma concentration of 5-azaCR would theoretically range from a high of 122 μg/ml with an injection of 100 mg/kg to a low of 9 μg/ml with a 72-hour infusion of a 50-mg/kg dose.

Toxicity was observed when 5-azaCR at a dose of 100 mg/kg was administered by injection and by the 6-hour infusion but not by the 72-hour infusion. The

TABLE 5.—Therapy for L1210 leukemic mice with combinations of 5-azaCR and THU by Alzet minipumps*

Dose of 5-azaCR† (mg/kg)	Ip infusion				72-hr infusion with Alzet minipumps			
	5-AzaCR‡	%ILS	5-AzaCR + THU‡	%ILS	5-AzaCR‡	%ILS	5-AzaCR + THU‡	%ILS
0 (control)	10.7 ± 2.7				10.7 ± 2.7		10.7 ± 2.7	
50	15.6 ± 2.6	46	16.5 ± 3.6	54	14.3 ± 1.2	37	14.7 ± 0.6	37
100	9.9 ± 5.7	Toxic	5.3 ± 4.5	Toxic	18.3 ± 2.3	71	15.0 ± 4.6	40

*Mice were inoculated on Day 0 with 1×10^5 leukemic cells and therapy was administered on Day 1. Controls received minipump plus saline or no treatment. Since the survival data for both controls were similar, the survival data were pooled.

†5-AzaCR and THU, where indicated, were administered in molar ratios of 5:1 (5-azaCR:THU).

‡Values are mean survival time (days) ± SD.

theoretic plasma concentrations of 5-azaCR with these toxic dose schedules were ≥ 122 μg/ml, whereas the same dose administered over 72 hours would have a plasma concentration of 19 μg/ml. If the plasma concentration of 5-azaCR were maintained at 61 μg/ml (50 mg/kg) for short periods or > 6 hours, toxicity was not observed. The administration of THU which might minimize the deamination of 5-azaCR and might maintain a greater concentration of 5-azaCR per se, was without effect on the toxicologic response to 5-azaCR. However, one cannot preclude that decomposition of 5-azaCR during the 72-hour infusion was not a contributing factor to the absence of toxicity.

In evaluating the therapeutic response to 5-azaCR, equivalent response was observed with a fourfold range in C×t, with a 13-fold range in the theoretic plasma concentration of 5-azaCR, and with a constant molar ratio of 5-azaCR and THU in plasma of 20:1. This equivalent response is surprising unless there may be a small percentage of cells within the population of L1210 cells which are refractory to 5-azaCR therapy. This rationale may explain the synergistic chemotherapy with combinations of 5-azaCR and BCNU.[6]

Our results show that the administration of THU to mice, prior to or simultaneously with 5-azaCR, markedly increased the urinary excretion of 5-azaCR and altered the urinary metabolite profile of 5-azaCR. On the other hand, minimal increases in plasma levels of both 5-azaCR and ara-C were observed when mice were given either drug ip in combination with THU (32,33). These data suggested to us that perhaps the major or only effect of THU is on the kidney cytidine deaminase. In addition, Camiener and Smith (35) showed that the mouse exhibited high pyrimidine nucleoside deaminase activity in the kidney while no measurable activity was found in liver, heart, or muscle. Thus, it appears likely that the kidney deaminase, more so than other tissue, may be responsible for the observed effect of THU on the urinary metabolite profile of 5-azaCR.

In summary, continuous infusion of 5-azaCR provides the potential for administering a greater total dose and attaining a greater C×t. Toxicologic response in mice occurred after exceeding a theoretic plasma concentration of 5-azaCR. However, chemotherapy did not appear to be related to total dose, C×t, plasma concentration, or schedule of administration of 5-azaCR and THU. It would suggest that a minimal amount of 5-azaCR is required to elicit a chemotherapeutic response and the absence of any additional therapy with a greater amount of drug is intriguing. A better understanding of this problem is needed in order to establish more meaningful chemotherapeutic regimens in the clinic.

[6] Arthur D. Little, Inc. Progress Report: Combination Chemotherapy, Combined Modality and Surgical Adjuvant Studies. Submitted to the DCT, NCI, Jan 1977, p 25.

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Effect of α-Tocopherol on the Cardiotoxicity of Adriamycin in the Rat[1,2,3]

P. Sonneveld[4,5,*]

SUMMARY

Adriamycin (ADR)-induced cardiomyopathy was studied in a rat model by means of electrocardiograms (ECGs), light microscopy, and determination of heart weight. Pretreatment with D-α-tocopherol (α-T) 24 hours prior to a high dose of ADR diminishes cardiotoxic effects, as judged by ECG changes, decreases in heart weight, and histologic changes. The response of an acute myeloid leukemia in a rat model to ADR was not affected by this pretreatment. The results provide evidence that α-T might be used to improve the therapeutic index of ADR.

[Cancer Treat Rep 62:1033–1036, 1978]

The anthracycline antibiotic adriamycin (ADR) is one of the most effective single agents in the treatment of malignant diseases (1). However, its clinical use has been limited by the occurrence of dose-dependent cardiotoxicity (2). It is now generally accepted that the maximum cumulative dose of ADR should not exceed 550 mg/m² (3). Previous studies in man and in animal models have shown variable histologic changes in the heart muscle fibers such as cytoplasmic vacuolization, myocytolysis, interstitial fibrosis, nuclear pyknosis, and reduction in periodic acid-Schiff (PAS) positivity (2–5). These cardiac lesions are similar to muscle lesions produced by an α-tocopherol (α-T) deficiency in the rabbit and the mouse (6,7). This α-T deficiency leads to lipid peroxidation of mitochondrial membranes due to free radical reactions. Lipid peroxidation can also be induced by drugs containing quinone and hydroquinone groups (8,9). Since ADR contains both quinone and hydroquinone groups, cardiac tissue damage caused by this drug could possibly be produced by mechanisms similar to those of the α-T deficiency. Myers et al (10) have recently demonstrated a reduction in ADR-induced mortality in CDF_1 mice after prior administration of α-T.

Such a protection against cardiotoxicity is only of value if the antitumor effect is not significantly reduced. ADR is presently widely employed in the treatment of acute myeloid leukemia (AML). Since results in the rat model are in agreement with clinical results, we have investigated the effects of pretreatment with α-T on the cardiotoxic and antitumor effects in our AML model in the Brown Norway (BN) rat (11,12).

MATERIALS AND METHODS

Eighty BN female rats (average weight, 200 ± 20 g), divided into 20 animals per group, were used. ADR was administered iv in 0.9% NaCl solution (2 mg/ml) at a dose of 7.5 mg/kg on Day 1 and 10 mg/kg on Day 15 (groups 2 and 3). Control animals received equivalent volumes of 0.9% NaCl solution iv (group 4). D-α-T was administered sc 24 hours prior to ADR in a dose of 3200 IU/kg, which is the equivalent of 2375 mg of vitamin E/kg (group 3). In group 1, α-T only was injected sc without following ADR treatment. α-T was dissolved in vegetable oil in a concentration of 320 IU/ml (groups 1 and 3). All groups received water and food ad libitum. Animals were weighed two times weekly. Electrocardiograms (ECGs) were recorded under light ether anesthesia on Days 0, 22, 31, and 38 (13). The precordial lead was used with a 20-mm standard. A decrease in QRS voltage was the only irreversible ECG change following long-term ADR administration in this rat strain. The mean deflection of the QRS voltage was calculated from the

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TABLE 1.—Normal rats treated on Days 1 and 15 with a total dose of ADR of 17.5 mg/kg*

Treatment group†	Heart weight (g ± SE)	Ratio (%)		Histologic changes (mean ± SD)
		Heart weight:body weight	Ascites and/or hydrothorax	
1. α-T	0.798 ± 0.027	0.34	0	0
2. ADR	0.548 ± 0.013	0.30	100	12.75 ± 0.94
3. α-T + ADR	0.644 ± 0.012	0.38	20	1.75 ± 0.32
4. Controls	0.902 ± 0.011	0.38	0	0

*Histologic changes are scored as incidence of edema, vacuoles, PAS positivity, and nuclear pyknosis from 0 to 4 each, in which the highest score represents the most severe change, and added. Values are mean ± SD; mean values of 20 animals/group are presented.

†α-T (3200 IU/kg) was given 24 hrs before ADR treatment; each group consisted of 20 rats.

measurements of all animals in each group. Animals were killed by ether anesthesia on Day 38 and inspected for the presence of hydrothorax and ascites. The hearts were removed and cleaned of fat, major vessels, and blood clots. Whole hearts were weighed and related to the total body weight. Light microscopic sections were prepared according to standard methods used in the Department of Pathology in the Institute for Experimental Gerontology (Dr. C. Zurcher). Sections were prepared on slides stained with both hematoxylin-phloxin-saffron (HPS) and diastase-PAS (D-PAS). HPS- and D-PAS-stained slides were investigated for the incidence of vacuoles, edema, PAS positivity, and nuclear pyknosis. Each of these changes was scored by a double-blind method using a scale of 0–4. The highest score indicated the most severe change. The maximum score that could be obtained in this system was 16 for each animal. The mean score for all animals in each group is presented in table 1.

The effect of α-T on the antitumor activity of ADR was studied in the BN acute myeloid leukemia (BNML) treated with cytosine arabinoside (Ara-C) and ADR at 16-hour intervals. This schedule consists of the administration of Ara-C (200 mg/kg iv) four times at 16-hour intervals followed 16 hours after the last dose by ADR (7.7 mg/kg iv) and is used in this Institute to induce remission in BNML rats. The schedule was begun on Days 15 and 35 after iv inoculation of 10^7 BNML cells. Survival with this schedule is about 50 days compared to 30 days for the same schedule without ADR.

RESULTS

ECG analysis (fig 1) showed no differences in QRS voltage between groups on Day 22. On Day 31, a significant decrease ($P < 0.005$) occurred in the ADR group; the α-T-pretreated ADR group showed no significant QRS decrease. On Day 38, a significant decrease ($P < 0.005$) in the QRS voltage was still seen in the ADR group. The α-T-pretreated rats showed no ECG changes when compared with controls. The group treated with α-T only showed a nonsignificant increase in QRS voltage on Day 38 when compared with controls, which was due to the high absolute values of three animals in this group.

After being killed (table 1), all animals in the ADR group and only four in the α-T-pretreated group showed pleural effusions. There was a heart weight decrease of 39% ± 2% (SE) in ADR-treated rats and 29% ± 1% (SE) in α-T-ADR-treated rats when compared with controls. The ratio between heart weight and body weight was calculated to quantify the relative change in heart weight. Table 1 shows a significant decrease in the ADR group, a nonsignificant decrease in the α-T group, and no decrease in the α-T-pretreated ADR group. In this table, scores of microscopic changes are indicated as means of each group. Group 1 and 4 animals showed no changes. In group 2, 18 rats exhibited histologic pathology, and in group 3 changes were detected in four animals.

Table 2 shows the influence of α-T on the antitumor activity of ADR in an AML drug-therapy schedule in the BNML rat which is used in this Institute. Sc administration of α-T (3200 IU/kg) 24 hours prior to ADR had no influence on survival time. No difference was observed in the mean body weight when animals died in either group. Liver and spleen weights, which are used as parameters for the stage of the leukemia and the effect of chemotherapy, showed no differences between groups with and without α-T pretreatment. Here, no hydrothorax was seen in the α-T-pretreated group but hydrothorax was present in three rats of the group receiving no α-T at the time of autopsy.

These results indicate that treatment with α-T

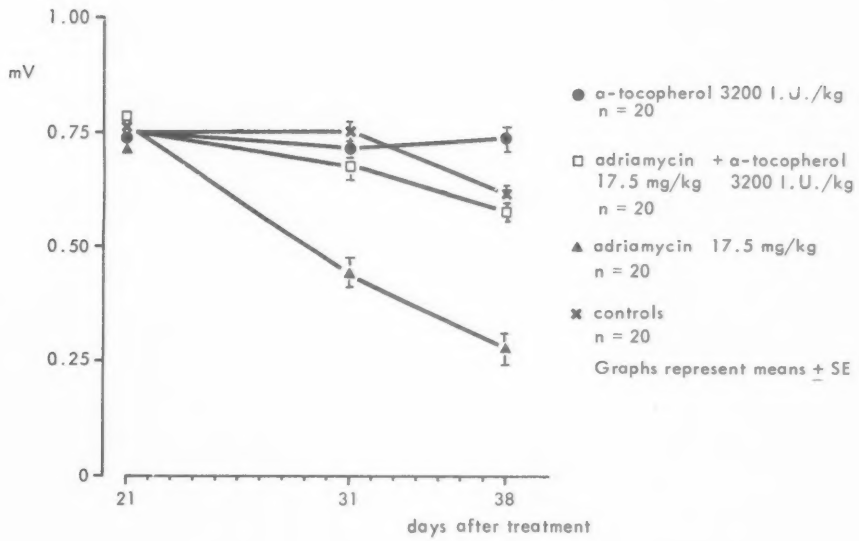

FIGURE 1.—ECG analysis.

significantly diminishes the dose-dependent cardiotoxicity of ADR in the rat without affecting the antitumor action of the drug. As suggested by Myers et al (8), α-T blocks the ADR-induced lipid peroxidation but not the binding of ADR to DNA, which seems to be the major cytotoxic action. Furthermore, these results suggest that the therapeutic index of ADR can be improved by use of larger doses, if dose-dependent cardiotoxicity is diminished by the administration of α-T. More detailed studies, including echocardiographic methods, with α-T in primates are in progress at this Institute to investigate the validity of this finding.

TABLE 2.—Effect of α-T on AML in the BN rat*

	ADR-Ara-C† (n = 10)	ADR-Ara-C + α-T† (n = 10)
Survival after inoculation in days	49.0 ± 2.4	49.3 ± 2.3
Heart weight (g)	0.59 ± 0.14	0.63 ± 0.09
Liver weight (g)	10.18 ± 0.8	9.92 ± 0.9
Spleen weight (g)	0.56 ± 0.10	0.56 ± 0.18
Ratio (%) of ascites and/or hydrothorax	30	0

*Treated with a 16-hr interval Ara-C and ADR schedule begun on Days 15 and 35 after iv inoculation of 10^7 BNML cells. Cumulative dose of Ara-C was 1600 mg/kg; cumulative dose of ADR was 15.4 mg/kg. α-T was administered 24 hrs before ADR.
†Values are mean ± SD.

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Sample Size Requirements for Evaluating a Conservative Therapy [1]

Robert Makuch* and Richard Simon [2]

SUMMARY

Determination of an adequate sample size for a clinical trial has traditionally involved the specification of type I (false positive) and type II (false negative) error rates, and a difference that one wishes to detect. Because newer therapy has generally been more invasive or more toxic, it is conventional for the type I error to be 0.05 in order that new therapy not be accepted as superior unless its advantages are definitively established. Recently, many new trials have been directed toward showing that a more conservative treatment is equivalent in efficacy to a standard intensive therapy. In this paper, we provide formulas which prescribe the sample size necessary to meet certain criteria specified by the investigator for this alternative type of clinical trial. In addition, the percent increase in total sample size is described when more patients are allocated to one treatment than the other.

[Cancer Treat Rep 62:1037–1040, 1978]

An important issue to be addressed in the planning stages of clinical trials is the determination of an adequate sample size. Traditionally, the investigator is required to specify a quantity D which represents the difference between the effect of two treatments which he regards as important and the type I and type II error rates associated with the statistical test which the investigator intends to apply. The test is a significance test of the null hypothesis that the two treatments are of equal efficacy. The type I error represents the probability of falsely rejecting the null hypothesis. The type II error indicates the probability of falsely accepting the null hypothesis when the true difference in efficacy is D. Within this context, many reports exist on sample size determination (1–8).

This formulation for testing the null hypothesis is appropriate in clinical trials which compare a new experimental treatment to a standard therapy. The new treatment is frequently more toxic and will be discarded unless it can be definitively demonstrated to be superior in efficacy to the standard treatment. Consequently, the type I error is generally specified as 0.05 whereas the type II error is often as great as 0.20. In oncology, the total sample size is often relatively small, eg, < 100 patients, because the new treatment will not be adopted unless the increase in efficacy (D) is great.

As treatments have improved, however, a new type of clinical trial has appeared in which this traditional formulation does not seem appropriate. Instead of being oriented toward detecting a significant difference between two treatments, many new trials are directed toward showing that a more conservative treatment is equivalent in efficacy to a standard intensive and toxic therapy. Some examples of such clinical trials include the comparison of: (*a*) abdominoperineal resection to electrofulguration for primary rectal cancer, (*b*) cranial irradiation plus intrathecal methotrexate to systemic methotrexate infusion as a prophylaxis for central nervous system leukemia, (*c*) combination chemotherapy to chlorambucil for metastatic nodular lymphomas, and (*d*) mastectomy to tylectomy for primary breast cancer.

Unlike the more traditional concepts underlying sample size determination, an objective of this type of clinical trial is the selection of the more conservative treatment when it is not worse than the standard treatment by more than some difference judged to be acceptable by the investigator. If an inadequately small sample size is used, the hypothesis of no difference in efficacy will often be erro-

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neously accepted by a significance test even though the true difference is considerable. This may result in the adoption of the new conservative treatment based upon an inadequate trial. Failure to reject the null hypothesis may not represent support for the conclusion that treatments are equivalent in efficacy. Because the type II error is not explicit in the performance or reporting of a significance test, this method of analysis in such situations invites false confidence in the result (a good example of this is seen in ref 9). If significance testing is to be used at all in this setting, it will often be necessary to use a significance level of 0.10 or 0.20 rather than the usual 0.05 to avoid the more dangerous false negative conclusion. For these reasons, the use of confidence intervals is the preferred method of analysis for such studies.

In this paper, we develop a method for determining an adequate sample size in evaluating a conservative therapy based on the confidence interval concept. Our development is therefore consistent with appropriate statistical practice for analyzing this type of study. Further, the sample size equations are formulated in a natural way, in order to lend insight to sample size determination. Although no new statistical principles are presented here, increased attention should be given to this practical medical situation since, from our experience, many investigators are unaware that large numbers of patients are often required to properly evaluate a conservative therapy.

METHODOLOGY

We shall assume that the primary measure of patient response is dichotomous (eg, complete remission or not, alive or dead after 2 years, "cured" or "not cured"). The probability of response for the intensive treatment is denoted π_1, and this probability for the new conservative treatment is denoted π_2. N_1 patients are assigned the first treatment and r_1 responses are observed. The observed response rate or success rate for the first treatment is therefore $p_1 = r_1/N_1$. Similarly, the values are N_2, r_2, and p_2 for the second treatment.

Based upon the patients studied, the best estimate of difference in treatment efficacy is (p_1-p_2). This observed difference is subject to random fluctuation, however. Thus, when the sample sizes N_1 and N_2 are small, even a small observed difference (p_1-p_2) is consistent with a large true difference in efficacy $(\pi_1-\pi_2)$. An approximate upper $100\cdot(1-\alpha)\%$ confidence limit for the true difference $(\pi_1-\pi_2)$ is $(p_1-p_2) + z_\alpha\cdot\sigma$, where z_α is the upper α tail point of the standard normal distribution (eg, $z_{0.05} = 1.645$) and σ is the standard error of (p_1-p_2):

$$\sigma^2 = \pi_1\cdot(1-\pi_1)/N_1 + \pi_2\cdot(1-\pi_2)/N_2. \quad [1]$$

The confidence statement can be written

$$\Pr[(\pi_1-\pi_2) > (p_1-p_2) + z_\alpha\cdot\sigma] = \alpha. \quad [2]$$

The confidence limit depends upon the observed difference (p_1-p_2) and the confidence level α, together with the true success rates π_1 and π_2, and the corresponding sample sizes N_1 and N_2. For infinite sample sizes, the observed difference (p_1-p_2) is an exact estimate of the true difference $(\pi_1-\pi_2)$ since σ approaches 0. In calculating the confidence limit at the end of the study, one can approximate σ using p_1 and p_2 to estimate the unknown π_1 and π_2.

The confidence limit is a random variable since it involves p_1 and p_2. It seems natural therefore to set the sample sizes sufficiently large so that, with a high probability $(1-\beta)$, the confidence limit will not exceed some specified value δ. This can be written

$$\Pr[(p_1-p_2) + z_\alpha\cdot\sigma > \delta] = \beta. \quad [3]$$

It follows that

$$\Pr\left[\frac{(p_1-p_2)-(\pi_1-\pi_2)}{\sigma} > \frac{\delta-(\pi_1-\pi_2)}{\sigma} - z_\alpha\right] = \beta. \quad [4]$$

This implies

$$\frac{\delta-(\pi_1-\pi_2)}{\sigma} - z_\alpha = z_\beta, \quad [5]$$

where z_β represents the upper β tail point of the standard normal distribution (eg, $z_{0.20} = 0.84$). If the study is designed so that an equal number of patients receive each of the two treatments ($N_1 = N_2 = N$), then the number of patients required for each treatment is readily seen to be

$$N = [\pi_1\cdot(1-\pi_1) + \pi_2\cdot(1-\pi_2)]\cdot\left\{\frac{z_\alpha + z_\beta}{\delta-(\pi_1-\pi_2)}\right\}^2 \quad [6]$$

from equations [1] and [5]. If there is no difference in treatment efficacy ($\pi_1 = \pi_2 = \pi$), then equation [6] simplifies to

$$N = 2\pi\cdot(1-\pi)\cdot\left\{\frac{z_\alpha + z_\beta}{\delta}\right\}^2. \quad [7]$$

This last equation specifies the required number of patients for each of the two treatments required to ensure with probability $(1-\beta)$ that the upper $100\cdot(1-\alpha)\%$ confidence limit for the true difference in

efficacy does not exceed δ. For planning purposes, the common success rate π is estimated for use in equation [7].

EXAMPLE

Consider designing a clinical trial to compare two surgical treatments for stage I adenocarcinoma of the female breast. The treatments are simple mastectomy and a more conservative tumor resection. The resection is much less debilitating, but will be considered acceptable only if it can be demonstrated with 90% confidence ($\alpha = 0.10$) that it is, at worst, 10% inferior to mastectomy ($\delta = 0.10$) with regard to 5-year survival rates. Naturally, one wishes that the resection is no worse, but this cannot be demonstrated statistically with a finite number of patients.

Suppose one accepts an 80% probability ($\beta = 0.20$) that the 90% confidence limit for a difference in efficacy does not exceed $\delta = 0.10$. Moreover, assume that data indicate 80% of clinical stage I patients are alive 5 years after a simple mastectomy ($\pi_1 = 0.80$). If it is anticipated that the true 5-year survival rate for resected patients is the same as for simple mastectomy patients, then equation [7] indicates that 144 patients are required for each of the two treatments. If it is anticipated that the 5-year survival rate for resected patients is only 75%, then equation [6] indicates that 624 patients are required for each of the two treatments. The number of patients is extremely large in this case because one is trying to rule out a difference of 10% when the true difference is 5%.

DISCUSSION

Recently, there has been an increase in the number of clinical trials designed to demonstrate a nonsignificant difference in treatment efficacy. This phenomenon reflects previous successes in the development of treatments, current activity in the development of new therapeutic modalities, new methods of treatment monitoring, and increased knowledge of biologic response mechanisms. Methods of sample size estimation for these studies are important in order that the results be conclusive. A confidence limit approach has been adopted here for sample size estimation since it is more informative to calculate and display the upper confidence limit of differences in true treatment efficacy for various confidence coefficients than to merely perform a single significance test of the null hypothesis.

In the determination of sample sizes, our equations bear superficial resemblance to those derived from a significance testing approach (ie, specifying a type II error of β to detect a true difference δ using significance level α). The two methods do differ, however. First, our design parameter δ represents the difference in treatment efficacy between a standard and conservative therapy that the investigator wishes to rule out with probability $(1-\beta)$. For clinical trials in which a conservative therapy is evaluated, the δ will be smaller than in traditional clinical trials which are usually designed to detect substantial increases in treatment efficacy. In addition, it is apparent that δ differs from $(\pi_1-\pi_2)$ which represents the true difference in efficacy between two treatments; the inclusion of both δ and $(\pi_1-\pi_2)$ in equation [6] explicitly characterizes these two underlying concepts in sample size determination. In practice, equation [7] will most often be used $(\pi_1-\pi_2 = 0)$ for sample size determination. Parameter values $\alpha = 0.10$, $\beta = 0.20$, and $\delta = 0.10$ will be reasonable for many studies.

Second, the numerical results involving sample size requirements differ between our approach and the significance testing method. As an example, if $\pi = 0.9$ and $\delta = 0.2$, then the sample size determined from the significance testing approach (*see* ref 10, pages 221–222) is 66.7% greater than that obtained from our equation [7]. In general, whenever the common probability of response π exceeds $(1+\delta)/2$, the sample size determined from equation [7] will be less than that using the significance testing approach. This range of π is to be expected in studies which attempt to evaluate a conservative therapy; if the success rate π is low, medical attention will instead focus on finding more efficacious therapies.

The results here can be directly applied to the case where the sample sizes for the two treatments are not equal. There are certain circumstances where unequal sample sizes may be desirable (11). It is generally recognized that having different numbers of patients on each of the treatments results in requiring a greater total number of patients, but the extent of the increase is not often quantified.

For the planning model considered here let there be k times as many patients on the conservative treatment as on the standard treatment ($N_2 = k \cdot N_1$, k may be $>$ or $<$ 1). It follows from equations [1] and [5] that

$$N_2 = [\pi_1 \cdot (1-\pi_1) + \pi_2 \cdot (1-\pi_2)/k] \cdot \left\{ \frac{z_\alpha + z_\beta}{\delta - (\pi_1 - \pi_2)} \right\}^2$$

and

$$N_1 = [k \cdot \pi_1 \cdot (1-\pi_1) + \pi_2 \cdot (1-\pi_2)] \cdot \left\{ \frac{z_\alpha + z_\beta}{\delta - (\pi_1 - \pi_2)} \right\}^2.$$

The total sample size ($N_1 + N_2$) is minimized with treatment groups of equal size, k = 1. If the success rates in the two groups are the same ($\pi_1 = \pi_2$), then a simple expression results for the fractional increase in total sample size caused by having unequal groups. This fraction is $f = (k-1)^2/4k$. If k = 2 (or 1/2), that is, a 2 to 1 randomization is used, then f = 1/8. Thus, 12.5% more total patients are required for a 2 to 1 randomization compared to an equal randomization. If a 3 to 1 randomization (k = 3 or 1/3) is considered, then f = 0.33, that is, one third more patients are needed than for an equal randomization. Randomizations more extreme than 2 to 1 are rarely desirable.

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cis-Dichlorodiammineplatinum(II) and Bleomycin in the Treatment of Esophageal Carcinoma [1,2]

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SUMMARY

Forty-seven patients with squamous cell carcinoma of the esophagus were treated with a combination of *cis*-dichlorodiammineplatinum(II) (*cis*-DDP) and bleomycin by infusion. *cis*-DDP at a dose of 3 mg/kg with mannitol and prehydration was given on Day 1. On Day 3, bleomycin was started as a 10–15-unit/m² loading dose followed by a 10–15-unit/m² 24-hour infusion for 4–7 days. Three groups of patients were treated: group 1 (no clinical evidence of metastatic disease) included 25 patients, all with no prior therapy; group 2 (measurable metastatic disease) included 13 patients, eight previously treated with surgery and/or radiation; group 3 (known nonmeasurable metastatic disease) included nine patients, all previously treated with surgery and/or radiation and/or chemotherapy. A second course of therapy was given on Day 28 to groups 2 and 3, and as soon after surgery as possible in group 1. Nineteen percent of patients had complete or partial responses with another 44% having minor regressions. Toxic effects were mainly renal effects, alopecia, nausea and vomiting, and stomatitis. There were two drug-related deaths. The combination of *cis*-DDP and bleomycin is useful in the treatment of patients with squamous cell carcinoma of the esophagus.

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Despite the use of aggressive surgery and radiation therapy, the prognosis for patients with squamous cell carcinoma of the esophagus remains poor. Overall, < 5% of these patients live for 5 years (1). Even in more selected groups, ie, patients able to undergo resection or curative radiation, 5-year survival rates range from 20% to 30% (2). Currently, the role played by chemotherapy is limited. Bleomycin, the best studied single agent, has been reported to give response rates of 0–60%; response duration is usually brief (3–9).

cis-Dichlorodiammineplatinum(II) (*cis*-DDP) has shown significant activity against testicular, bladder, and head and neck cancers (10–12). At the Memorial Sloan-Kettering Cancer Center (MSKCC), 70% of previously untreated patients with squamous cell carcinoma of the head and neck showed major objective responses to a combination of *cis*-DDP and bleomycin (13). We were encouraged to use a similar combination in patients with squamous cell carcinoma of the esophagus. This is a preliminary report on the results of a continuing study.

MATERIALS AND METHODS

Forty-seven patients with histologic or cytologic proof of squamous cell carcinoma of the esophagus were treated. All slides were reviewed and the diagnosis was confirmed by the Department of Pathology of MSKCC. The median age was 58 years (range, from 31 to 76 years). Fifty-seven percent of the patients were men. Seventy-eight percent were white, 20% were black, and there was one patient of Japanese ancestry. One patient had been previously treated with 5-fluorouracil; no other patient had received prior chemotherapy. Three patients had been previously treated with radiation therapy

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alone, ten with both surgery and radiation, and four with surgery alone. Thirty patients had received no prior therapy. The primary tumor was located in the upper third of the esophagus in ten patients (21%), the middle third in 19 (40%), and the lower third in 18 (39%). Ninety-seven percent of the patients had a history of heavy smoking; 66% had a history of heavy alcohol ingestion. Six patients had had prior squamous cell carcinomas of the head and neck area.

Prior to therapy, the following studies were obtained: history and physical examination, cbc count and differential, platelet count, chest roentgenogram, barium esophagram, urinalysis, 24-hour urine for creatinine clearance, serum BUN and creatinine, SMA–12, complete pulmonary function tests and arterial blood gas tensions, and an electrocardiogram. An audiogram was performed if auditory loss was suspected by history or physical examination. Baseline studies were repeated prior to each course of therapy or prior to surgery in the preoperative group. Each course of *cis*-DDP and bleomycin was given in the hospital. Serum creatinine and cbc and platelet counts were repeated frequently during each course of therapy.

cis-DDP[4] was supplied by the Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md) in vials containing 10 mg of sodium chloride, 100 mg of mannitol, and 10 mg of *cis*-DDP. It was reconstituted with sterile water, 10 ml/vial, and infused within 1 hour of reconstitution. All patients received prehydration and a mannitol-induced diuresis according to a protocol developed at this institution (14). The dosage of *cis*-DDP was 3 mg/kg given as a rapid iv infusion (over 15–20 minutes) on Day 1.

Bleomycin (Blenoxan, Bristol Laboratories, Syracuse, NY) was given initially as a 15-unit/m² rapid iv push loading dose on Day 3 followed by an iv infusion of 15 units/m² in 1000 ml of 5% dextrose in water over 24 hours for 6–7 days. Ten patients received this dosage. In order to allow more frequent courses while keeping the total dose per year in a safe range, subsequent patients received a 10-unit/m² loading dose and a 10-unit/m² 24-hour infusion from Days 3 to 6. A loading dose was given in order to achieve a more rapid steady state blood level. If, prior to any course of therapy, the creatinine clearance dropped to < 60 ml/minute, *cis*-DDP was not given during that course. If the vital capacity declined by > 25% of the pretreatment value, bleomycin was not given during that course. The development of mucositis during treatment was an indication to stop the bleomycin infusion. The second course of therapy was given on Day 28; subsequent courses were given every 6–8 weeks. In the pre-operative group, the second course of therapy was given as soon after surgery as possible, generally 12–14 days.

Patients were divided into three groups. Group 1 (no clinically evident metastatic disease) included 25 newly diagnosed patients, all without prior therapy. Twenty-one of these patients subsequently underwent surgery. Four patients were felt not to be surgical candidates because of non-tumor-related medical problems. These four patients received radiation therapy after one (two patients), two (one patient), or three (one patient) courses of chemotherapy. Group 2 (measurable metastatic disease) included 13 patients, eight previously treated with surgery and/or radiation therapy. Group 3 (known nonmeasurable metastatic disease) included nine patients, all previously treated with surgery and/or radiation and/or chemotherapy.

Previously untreated patients who were operative candidates received a single course of chemotherapy followed by surgery on Days 14–18 following completion of the bleomycin infusion (fig 1). Evaluation of response to chemotherapy was by repeat barium esophagram performed on Days 18–21 (immediately prior to surgery). Patients were staged according to the staging system of the American Joint Committee for Cancer Staging and End Results Reporting (15). Stage I and II patients received a second course on Days 10–14 postoperatively. Subsequent courses were given every 6–8 weeks. In stage III patients, the second course of chemotherapy was followed by radiation therapy. Four hundred rads of radiation therapy were given twice a week for 4 weeks. Subsequent courses of chemotherapy were given starting 2 weeks after completion of radiation therapy and then every 6–8 weeks.

Thirteen patients in groups 2 and 3 had previously received radiation therapy. No patient received radiation therapy within 30 days of starting chemotherapy. Fifteen patients received nutritional support by total or partial parenteral nutrition.

Complete remission (CR) was defined as resolution of all measurable disease by roentgenogram, radionuclide scan, and physical examination for at least 1 month. In group 1 patients, no viable tumor could be found at surgery. Partial remission (PR) was defined as a decrease of > 50% in the sum of the product of the two longest perpendicular diameters of measurable masses for at least 1 month. Minor responses were defined as decreases of < 50% in the sum of the product of the two longest perpendicular diameters of measurable masses. Esophagrams

[4]Manufactured by the Ben-Venue Corp, Bedford, Ohio.

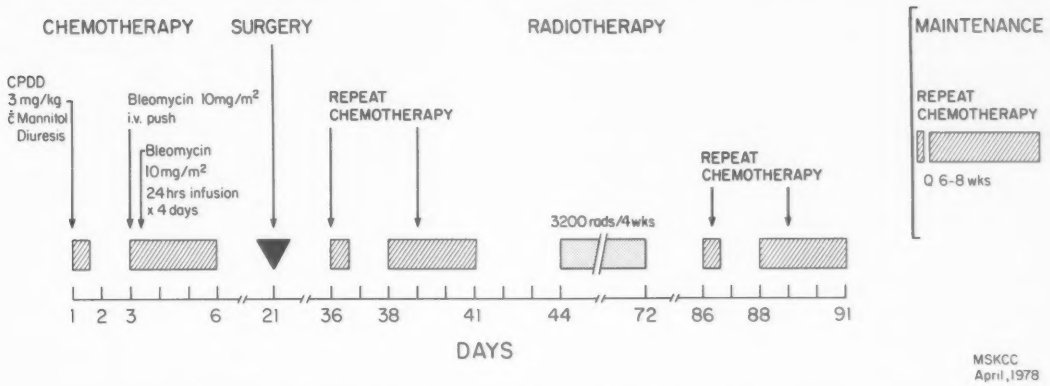

FIGURE 1.—Combined therapy approach in previously untreated patients.

were evaluated by visual estimation of reduction of tumor mass: CR, 100% resolution of tumor; PR, > 50% reduction in tumor; minor response, < 50% reduction in tumor. Complete resolution on barium esophagram required endoscopic confirmation to be called a CR.

Informed consent was obtained from all patients prior to the start of therapy. No patient with acceptable renal and auditory function was denied therapy because of poor performance status.

RESULTS

Forty-seven patients were treated. Eighteen patients received one course, 19 received two courses, and ten received three or more courses of chemotherapy. Nine patients in group 3 (nonmeasurable metastatic disease) were not evaluable for objective response and are being followed for survival duration. One patient in group 2, who died from disease during the first course of therapy, was considered inevaluable for response.

Thirty-seven patients were evaluable for objective tumor response: 25 in group 1 and 12 in group 2. Responses are shown in table 1.

In group 1 patients (evaluated on Days 18–21), there were four PRs and ten minor regressions. Duration of response in group 1 patients cannot be ascribed to chemotherapy alone. Of the 21 patients in group 1 who underwent surgery, 11 had demonstrated objective responses by Days 18–21. Twelve of 21 patients had resectable disease; eight of these had PR or minor regression to pre-operative chemotherapy. Of the 12 patients who had resection, eight are alive without evidence of disease 4+–16+ months from onset of therapy. Two of the nine patients who had unresectable disease are alive with disease at 3+ and 7+ months (one is terminally ill at this time). All responses in group 1 patients were evaluated by serial esophagrams.

TABLE 1.—Response

	No. of patients*	
	Group 1	Group 2
CR	0	1 (9.5)
PR	4	2 (6, 5+)
Minor regression	10	6 (1, 2, 3, 3, 3+, 4+)
No response	11	3
Totals	25	12

*Nos. in parentheses = response duration in mos.

In group 2 patients, there was one CR, two PRs, and six minor regressions. Response duration is shown in table 1. Responses of group 2 patients were evaluated by esophagram in three, esophagram and lymph node metastases in four, lymph node metastases alone in three, esophagram and pulmonary metastases in one, and tracheostomy recurrence in one. Responses were seen in the esophagus, lymph nodes, and pulmonary metastases.

Overall, including groups 1 and 2, responses were: esophagus, four PRs and 12 minor regressions; lymph nodes, one CR and one PR; lymph nodes and esophagus, one PR and three minor regressions; and pulmonary metastases and esophagus, one minor regression. In patients in group 2 achieving CR

TABLE 2.—Toxicity

Toxic effect	% of patients
Myelosuppression	
Wbc count < 2000/mm³	5
Platelet count < 50,000/mm³	9
Nausea and vomiting	67
Diarrhea	33
Stomatitis	17
Skin changes	15
Alopecia	70
Renal (serum creatinine ≥ 2.5 mg/100 ml)	13
Pulmonary (symptomatic)	4
Ototoxic effects	13
Transient tinnitus	22
Chills	15
Fever	33

or PR, maximum extent of response was achieved after two courses of chemotherapy.

Survival duration from onset of chemotherapy in group 2 (12 patients) ranged from 1 to 11 months (median, 4 months) with four patients still alive at 4+, 4+, 5+, and 6+ months. In group 3 (nine patients), survival ranged from 1 week to 6 months, with two patients surviving at 4+ and 6+ months. The four survivors in group 2 had all demonstrated objective responses to chemotherapy.

Toxicity

Toxicity data were evaluable in 45 patients (table 2). Alopecia, which was observed in 70% of patients, was almost total in many and was completely reversible at the end of treatment.

Nausea and vomiting occurred in more than two thirds of patients and was generally moderate to severe. Some patients had nausea and vomiting during one course of therapy but not during another, and it was our impression that, generally speaking, it was less marked than in patients with other diseases treated with *cis*-DDP.

Myelosuppression was generally mild with only 5% of patients having wbc counts fall to < 2000/mm³, and only 9% having platelet counts fall to < 50,000/mm³. In those courses in which the wbc count fell to < 4000/mm³ and the platelet count to < 150,000/mm³, the nadir of the wbc count depression occurred on Day 10 (range, Days 4–17). One patient required a platelet transfusion. Infection, especially pneumonia secondary to recurrent aspiration or tracheoesophageal fistula, was seen in 10% of patients, but was not related to drug-induced myelosuppression.

Renal toxic effects (peak serum creatinine ≥ 2.5 mg/100 ml) were seen in six (13%) patients. In these six patients, three had peak posttreatment creatinine levels of 2.5–3.0 mg/100 ml, which subsequently returned toward normal (serum creatinine, < 2.0 mg/100 ml). One patient had a peak serum creatinine of 5.4 mg/100 ml, which in 2 weeks fell to 1.9 mg/100 ml. Two patients died of renal failure: one with far-advanced metastatic disease, a pretreatment Karnofsky performance status of 30, and a creatinine clearance of 100 ml/minute, and the second with a performance status of 80, metastatic disease, and a pretreatment creatinine clearance of 60 ml/minute. In the one patient receiving five courses of *cis*-DDP, a slow rise of serum creatinine from a baseline level of 0.6 mg/100 ml to 1.4 mg/100 ml over 7 months was noted.

Clinically, ototoxicity was mild and did not interfere with subsequent courses of *cis*-DDP. Skin changes (hyperpigmentation and striae) and stomatitis were seen in 15% and 17% of patients respectively, but were not a major problem. Stomatitis developed after completion of the bleomycin infusion, usually 2–3 days after, and generally lasted for 1 week. Topical anesthetics (eg, Xylocaine viscous) were prescribed as needed.

In seven patients, bleomycin was withheld for one or more courses because of a > 25% decrease in vital capacity after previous courses of therapy. In two of these patients, decreased vital capacity, noted after thoracotomy, subsequently returned toward baseline values, allowing continuation of bleomycin therapy. Two patients developed respiratory symptoms, one with clinically significant pulmonary impairment; the other five patients were asymptomatic.

DISCUSSION

In an earlier study at MSKCC, 70% of previously untreated patients with squamous cell carcinoma of the head and neck showed major objective responses to the combination of *cis*-DDP and bleomycin. Toxicity in this group was similar to that seen in the present study. Because the promising initial results of the combination in head and neck cancer appeared superior to bleomycin alone, *cis*-DDP and bleomycin were used in patients with esophageal cancer. Since bleomycin seems to have some activity in esophageal cancer, and since *cis*-DDP and bleomycin have different toxic effects allowing full doses of both to be used, we decided to use the combination rather than *cis*-DDP alone.

The present study has demonstrated CRs and PRs to a combination of *cis*-DDP and bleomycin in 19% of patients with squamous cell carcinoma of

the esophagus. An additional 44% of patients had minor regressions; all patients with minor regressions as measured by esophagram had marked symptomatic improvement in swallowing function. Those patients in group 2 with CRs and PRs tended to have a longer duration of response and survival than did patients with minor regressions. The survival of patients with minor regressions or with no response to chemotherapy was similar. Major toxic effects were nephrotoxic effects, alopecia, and nausea and vomiting; clinically significant pulmonary toxic effects were seen in only 4% of patients. In general, the combination was reasonably well-tolerated.

Despite innovative surgical techniques and widespread use of supervoltage radiation, the prognosis for patients with squamous cell carcinoma of the esophagus remains poor. The 5-year survival rate for all patients is low (2). Forty percent of patients are inoperable on initial presentation; of those having operations, only 50%–60% have resectable disease (15,16). Clearly, improvement in survival, as well as in palliation, would be aided by efficacious chemotherapy.

Bleomycin is the best studied single agent in esophageal carcinoma. In previous studies, bleomycin has been given by rapid iv or im injection, rather than by infusion as in our study. Reported response rates have ranged from 0 to 60%. Tancini and Bonadonna et al (3,4) have reported responses of 50%–60% with a median duration of response of 1–2 months. Although their criteria of response were not identical to ours, 14%–20% of their patients appear to have had CRs or PRs. In a comparison of bleomycin alone and bleomycin plus radiation therapy, Kolarić et al (7) reported four responses among 15 patients treated with bleomycin alone. The median duration of response was 2.6 months. On the other hand, Ravry et al (8) had no responders in 14 patients. In other studies involving bleomycin, responses and response durations were not quantitated, making it difficult to interpret the results. Folke (5) reported a series of 21 African patients receiving bleomycin as a single agent. Response to therapy was assessed by symptomatic improvement in swallowing; the followup was incomplete. The extensive Japanese experience has been summarized by Nabeya (6). Criteria of response and duration of response were not defined. Occasional responses, usually in drug-oriented bleomycin trials, have also been reported (9,17,18). It appears that bleomycin as a single agent has limited activity in patients with squamous cell carcinoma of the esophagus. Median durations of response in studies with bleomycin as a single agent range from 1 to 2.6 months.

Our criteria of response appear substantially stricter than those of most investigators. For example, we require endoscopic or surgical confirmation of CR as measured by esophagram. Complete and partial responders in group 2 had easily measurable lymph node metastases. Thus, although several studies of bleomycin used as a single agent reported CR or PR rates of 14%–20%, their criteria of response do not appear to have been as rigid as in the present study. In addition, since it took two courses of *cis*-DDP and bleomycin to reach the maximum extent of response in group 2 patients, it is possible that at least some of the ten minor regressions in group 1 patients might have become PRs after a second course of chemotherapy. In complete and partial responders in group 2, the median duration of response will be at least 6 months. Toxic reactions in most patients have been easily managed. A prospective, randomized controlled study of bleomycin and *cis*-DDP plus bleomycin would test the apparent superiority of the combination.

The combined modality approach in group 1 patients is currently under study. This approach involves close cooperation between the Thoracic Surgery, Solid Tumor, Radiation Therapy, and Nutrition Services at MSKCC. The initial results of combination therapy, partly presented in this report, are encouraging. Further work is needed before recommending *cis*-DDP and bleomycin as an adjunct to surgery in patients with esophageal carcinoma.

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Antitumor Properties of a New Folate Analog, 10-Deaza-aminopterin, in Mice [1,2]

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SUMMARY

A new folate analog, 10-deaza-aminopterin, was substantially more active than methotrexate, following sc administration in mice, against three of five ascites tumors and two of three solid tumors. For ascites tumors, maximum increases in lifespan (using 6–12 mg/kg q2d × 5) with 10-deaza-aminopterin versus methotrexate were +171.2%/+149.8% against L1210 leukemia, +118.4%/+109.1% against P815 plasmacytoma, +64%/+20.9% against Ehrlich ascites carcinoma, +84.2%/+44.8% against Taper liver tumor, and >+159.6%/+64.0% against Sarcoma 180 with long-term survivors after 10-deaza-aminopterin. In a smaller number of experiments comparing LD10 dosages (given q2d × 5) of aminopterin, methotrexate, and 10-deaza-aminopterin, aminopterin was the least effective and 10-deaza-aminopterin was the most effective against the L1210 leukemia, Sarcoma 180, and Ehrlich ascites tumors. Following oral administration (3–6 mg/kg q2d × 5), a twofold greater increase in survival time was obtained against the L1210 leukemia with 10-deaza-aminopterin (+122.8%) versus methotrexate (+57%). At a dosage of 6 mg/kg q1d × 5 against solid tumors, the relative tumor volumes (treated/control × 100%) were 12%/41% for Sarcoma 180, 16%/31% for Taper liver tumor, and 20%/30% for Ehrlich ascites carcinoma. Overall, the data suggest a broader spectrum of effective antitumor action in mice and a potential for the expanded clinical utility of this category of agent.

[Cancer Treat Rep 62:1047–1052, 1978]

The antitumor efficacy of the folate analog, methotrexate, has been well documented in some experimental neoplasms (1–4) and in certain forms of human cancer (5–7). In patients, both acute lymphocytic leukemia and choriocarcinoma are two prominent examples of tumors highly susceptible to this agent. However, other human cancers, for the most part, are either only marginally responsive or not susceptible at all.

In an effort to develop analogs more effective against a broader range of human cancers, we have sought information on the structural basis for relative responsiveness, toxicity, and selective action of the general category of folate analogs. Evidence gathered so far at both biochemical (8–10) and pharmacologic (11,12) levels strongly suggests that, at least in murine tumor models, the degree of selective action is determined at the level of membrane transport. Some correlation between uptake of methotrexate and responsiveness of a group of murine tumors was also reported in an earlier study (13) done elsewhere. In the case of methotrexate, the relatively high degree of selective antitumor action observed was associated (10) with a difference (in tumor versus drug-limiting normal tissue) in the specificity for N^{10} substituents favoring greater levels of accumulation and persistence of drug in tumor tissue. Consequently, our initial search for new agents has focused on analogs altered at the N^{10} position. In the present study, we evaluate the

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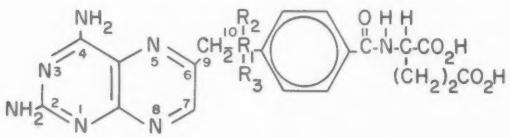
antitumor activity of such an analog, 10-deaza-aminopterin, compared to both methotrexate and aminopterin against a variety of ascitic and solid murine tumors, some of which are highly predictive of responsiveness in human cancer. The data presented clearly document the superiority of 10-deaza-aminopterin over the other two analogs in three nonlymphoidal tumors.

MATERIALS AND METHODS

Ascitic forms of the L1210 leukemia, Sarcoma 180 (obtained from Dr. G. Chihara, National Cancer Institute, Tokyo, Japan), P815 plasmacytoma, Ehrlich ascites carcinoma, and Taper liver tumor were maintained (4) by transplantation ip in female $BD2F_1$ mice (C57BL female/DBA_2 male obtained from A. R. Schmidt, Madison, Wis). For all experiments, new lines were initiated in $BD2F_1$ mice from a bank of frozen cells prepared earlier from a line determined to exhibit characteristic malignancy and maintained for only five transplantation generations and then discarded. The synthesis and antifolate properties in bacteria of 10-deaza-aminopterin have been described earlier (14). All three folate analogs (*see* fig 1 for structural formulas) were prepared as the sodium salt in neutral aqueous solution and usually administered sc in the scapular region. In some experiments, drug was administered orally by gastric needle. Therapy was initiated 24 hours after tumor transplantation in animals (20 g) randomly selected for control (untreated) and treated groups in each experiment. During therapy against the ascites tumors, mice were inoculated ip with 10^6 tumor cells (0.1 ml). Untreated animals died with well-developed ascites within 6–8 days (L1210), 10–12 days (Sarcoma 180), 7–9 days (P815), 16–20 days (Ehrlich), and 14–18 days (Taper). During therapy against the solid tumors, 10^6 ascites tumor cells (0.05 ml) were implanted intradermally in the flank after removing the coat with an electric clipper. After the start of therapy, the average tumor diameter was 2 mm. This increased in untreated control groups to 8 ± 2 mm in 7 days. During the total period of the experiment, the tumor-bearing animals did not develop ascites or exhibit any evidence of metastatic tumor. The average tumor diameter in millimeters was determined from measurements along two axes of the tumor and the tumor volume was calculated from the equation, $V(mm^3) = 4/3\ \pi r^3$. The data are expressed as relative tumor volume (%) by means of the following calculation: $T\ (V_t - V_0) \times 100$, where T = treated group, C = control group, V_0 = volume at the time of treatment, and V_t = volume after treatment. Animals were maintained with Purina laboratory chow and water ad libitum.



	R_1	R_2	R_3
aminopterin	N	H	—
methotrexate	N	CH_2	—
10-deaza-aminopterin	C	H	H

FIGURE 1.—Structural formulas for 10-deaza-aminopterin, aminopterin, and methotrexate.

RESULTS AND DISCUSSION

The antitumor effects of 10-deaza-aminopterin and methotrexate against a group of transplanted murine ascites tumors are compared in table 1. The drug was given sc on a schedule of one dose every 2 days for a total of five doses. This route of drug administration avoids the direct exposure of the tumor implant (ip) to drug and was employed in order to provide the most valid comparison of the two agents which differ (15) only in their membrane transport properties. The antitumor effects observed were dose-dependent within approximately the same dose range (3–18 mg/kg) for each agent. However, 10-deaza-aminopterin was slightly more toxic than methotrexate, as indicated by the greater decrease in average weight compared to controls at the time of death at doses > 9 mg/kg. Greater therapeutic effects (increase in lifespan) were obtained with 10-deaza-aminopterin against all of the tumors tested. Differences were smallest in the case of L1210 leukemia, the most responsive tumor, and P815 plasmacytoma. However, the effects of 10-deaza-aminopterin were far greater than for methotrexate in the case of the other three tumors. The most interesting result was seen with Sarcoma 180 where the maximum antitumor effects obtained with the new analog were two- to three-fold greater than that obtained with methotrexate and reached levels of response observed against the L1210 leukemia. Increases in lifespan in one of the three experiments carried out with Sarcoma 180 exceeded +220% for 10-deaza-aminopterin with long-term survivors versus +83% for methotrexate. Relative effects of this new analog compared to methotrexate against both Ehrlich ascites carcinoma (threefold) and Taper liver tumor (twofold) were similar to that seen with Sarcoma 180. The overall level of response of these tumors, however, was less.

The antileukemic superiority of 10-deaza-aminopterin versus methotrexate following oral administration is documented by the results shown in table 2. The analogs were given to L1210 leukemia-bearing mice once every 2 days for a total of five doses. A comparison of the data on average final weight obtained at each dosage suggests somewhat greater toxicity for both agents by this route of administra-

TABLE 1.—Relative efficacy of sc administered methotrexate and 10-deaza-aminopterin against a variety of murine ascites tumors

Tumor	Drug	No. of mice*	Dose† (mg/kg)	MST ± SD (days)	ILS (%)	Average weight‡ (g)
L1210	—	5 × 4	—	6.7 ± 0.8	—	23.1 ± 0.6
	Methotrexate	5 × 2	3	11.5 ± 1.1	71.8	22.8 ± 0.9
		5 × 2	6	14.4 ± 0.8	114.9	22.6 ± 1.9
		5 × 3	9	15.8 ± 0.9	136.0	23.0 ± 1.5
		5 × 3	12	16.7 ± 1.1	149.8	22.2 ± 1.8
		5 × 4	18	15.8 ± 1.9	136.0	18.3 ± 2.4
	10-Deaza-aminopterin	5 × 4	3	13.8 ± 0.7	106.2	22.5 ± 1.7
		5 × 2	6	15.2 ± 1.5	127.0	23.1 ± 2.1
		5 × 3	9	17.9 ± 0.8	167.8	21.5 ± 2.3
		5 × 3	12	18.2 ± 1.3	171.6	20.3 ± 2.3
		5 × 2	18	17.9 ± 1.6	167.8	17.2 ± 2.7
Sarcoma 180	—	5 × 3	—	12.1 ± 1.1	—	28.4 ± 2.7
	Methotrexate	5 × 2	3	11.7 ± 1.7	0	26.2 ± 2.0
		5 × 3	6	15.2 ± 0.9	24.9	29.0 ± 3.1
		5 × 3	9	18.3 ± 2.0	51.3	29.4 ± 2.8
		5 × 3	12	19.8 ± 1.8	64.0	27.2 ± 3.2
		5 × 2	18	19.4 ± 2.7	60.6	19.6 ± 4.1
	10-Deaza-aminopterin	5 × 2	3	15.7 ± 1.1	28.7	26.0 ± 2.2
		5 × 3	6	21.2 ± 1.7	74.8	28.6 ± 1.9
		5 × 4	9	27.2 ± 0.9	124.3	27.2 ± 2.6
		5 × 3	12	> 31.4 ± 2.5	>153.6§	24.6 ± 2.9
		5 × 2	18	22.1 ± 2.4	84.0	16.8 ± 3.2
P815 plasmacytoma	—	5 × 2	—	8.8 ± 0.9	—	22.4 ± 1.2
	Methotrexate	5 × 2	6	15.4 ± 1.1	75.0	23.4 ± 0.6
		5 × 2	9	17.6 ± 1.6	100.3	22.7 ± 1.7
		5 × 2	12	18.4 ± 1.3	109.1	22.2 ± 0.9
	10-Deaza-aminopterin	5 × 2	6	18.2 ± 1.3	107.5	23.1 ± 0.8
		5 × 2	9	19.0 ± 0.9	116.3	22.4 ± 1.3
		5 × 2	12	19.2 ± 1.2	118.4	18.6 ± 1.8
Ehrlich ascites	—	5 × 2	—	17.2 ± 2.1	—	31.2 ± 1.9
	Methotrexate	5 × 2	6	19.8 ± 2.3	15.1	31.0 ± 2.8
		5 × 2	9	20.8 ± 3.0	20.9	30.4 ± 3.4
		5 × 2	12	20.2 ± 1.9	17.0	29.6 ± 3.2
	10-Deaza-aminopterin	5 × 2	6	23.2 ± 1.8	34.8	30.6 ± 2.4
		5 × 2	9	27.2 ± 2.2	58.1	32.0 ± 4.1
		5 × 2	12	28.2 ± 2.9	64.0	25.6 ± 3.1
Taper liver	—	5 × 2	—	14.2 ± 2.2	—	32.4 ± 1.8
	Methotrexate	5 × 2	6	19.7 ± 2.3	39.0	29.3 ± 2.7
		5 × 2	9	20.6 ± 2.8	44.8	29.7 ± 4.3
		5 × 3	12	20.2 ± 3.1	41.6	30.4 ± 3.8
	10-Deaza-aminopterin	5 × 3	12	26.1 ± 2.9	84.2	29.5 ± 4.8

* No. of mice in each experiment × No. of experiments.
† Given as a single sc injection.
‡ Initial weight = 20 g.
§ Two 60-day survivors.

tion. It should be pointed out that this appears to be a real difference between orally and parenterally administered drug, since some of the results of these experiments were derived in parallel with the results shown in table 1. Antileukemic effects were generally lower following oral administration of drug (compare tables 1 and 2). However, the maximum effect obtained with 10-deaza-aminopterin was twofold greater than for methotrexate.

Another, but more limited, study of the relative antitumor effects of 10-deaza-aminopterin versus methotrexate was carried out for the purpose of obtaining a comparison with aminopterin. All three agents were given q2d × 5 at the approximate LD10 for this schedule. These dosages were determined by a preliminary toxicity assay. The relative therapeutic effects on this schedule against the L1210 leukemia, Sarcoma 180, and Ehrlich ascites carcinoma tumors are given in table 3. The data show an overall level of responsiveness for each tumor similar to that shown in table 1. In every case, 10-deaza-aminopterin was the most effective and aminopterin was the least effective agent. Again, the best result with 10-deaza-aminopterin was observed with Sarcoma 180.

A comparison of the effects of 10-deaza-aminopterin and methotrexate against solid tumors is shown in table 4. Both analogs were tested at a dose of 6 mg/kg sc which resulted in some toxicity on the schedule employed (q1d × 5). 10-Deaza-aminopterin was clearly more effective than methotrexate against solid forms of Sarcoma 180 and Taper liver tumor. The difference in the effect of both agents was similar to that observed against the corresponding ascites tumors (table 1). Only a small difference in therapeutic effect in favor of 10-deaza-aminopterin could be seen with the Ehrlich ascites carcinoma.

In a preliminary biochemical and pharmacologic study reported earlier (15), 10-deaza-aminopterin was found to be indistinguishable from methotrexate with respect to inhibition of dihydrofolate reductase and rate of plasma clearance. The increased therapeutic efficacy of the new analog over methotrexate now reported here can be associated (15) with a greater persistence of exchangeable levels of this agent in tumor but similar persistence of each in drug-limiting normal proliferative tissue in the small intestine. Larger differences in the persistence of 10-deaza-aminopterin versus methotrexate in Sarcoma 180 were accounted for by differences in membrane transport, specifically, the saturability (Michaelis constant) of influx, favoring greater accumulation of 10-deaza-aminopterin. Both analogs competed for the same carrier transport mechanism in tumor and normal tissue, but carrier in tumor exhibited a much greater affinity (three- to fourfold) for 10-deaza-aminopterin. These data (15) and the results presented here would seem to suggest a broader spectrum of clinical potential for 10-deaza-aminopterin compared to methotrexate and provide justification for a further period of rational design

TABLE 2.—Relative efficacy of methotrexate and 10-deaza-aminopterin following oral administration to L1210 leukemic mice

Drug	No. of mice*	Dose† (mg/kg)	MST ± SD (days)	ILS (%)	Average weight ± SD‡ (g)
—	5 × 4	—	6.6 ± 0.7	—	22.3 ± 0.7
Methotrexate	5 × 3	0.75	7.7 ± 0.9	15.2	21.8 ± 0.6
	5 × 4	1.5	7.9 ± 0.6	18.9	23.2 ± 0.7
	5 × 4	3	9.4 ± 1.1	41.7	23.1 ± 0.5
	5 × 4	4.5	9.6 ± 0.8	45.1	22.6 ± 0.8
	5 × 4	6	10.4 ± 0.7	57.8	21.9 ± 2.0
	5 × 2	9	10.2 ± 1.3	54.0	20.3 ± 1.9
	5 × 2	12	10.3 ± 1.7	55.3	18.4 ± 1.7
10-Deaza-aminopterin	5 × 3	0.75	8.9 ± 1.2	34.2	22.4 ± 1.0
	5 × 4	1.5	10.4 ± 0.8	57.5	22.8 ± 0.4
	5 × 4	3	11.7 ± 1.0	77.2	22.7 ± 1.2
	5 × 4	4.5	13.3 ± 0.9	101.3	23.3 ± 0.9
	5 × 4	6	14.7 ± 1.3	122.8	21.4 ± 1.8
	5 × 2	9	12.1 ± 1.8	84.4	19.2 ± 2.2
	5 × 2	12	10.9 ± 1.9	66.6	16.8 ± 2.1

* No. of mice in each experiment × No. of experiments.
† Given as a single oral injection.
‡ Initial weight = 20 g.

TABLE 3.—Relative efficacy of sc administered methotrexate, aminopterin, and 10-deaza-aminopterin at equitoxic (LD10) dosage schedules

Tumor	Drug	No. of mice*	Dose† (mg/kg)	MST ± SD (days)	ILS (%)	Average weight ± SD‡ (g)
L1210	—	5 × 4	—	6.6 ± 0.4	—	22.4 ± 0.4
	Aminopterin	5 × 4	0.23	10.9 ± 0.8	80.5	21.5 ± 0.5
	10-Deaza-aminopterin	5 × 2	9.0	18.3 ± 1.9	178.2	21.0 ± 1.4
	Methotrexate	5 × 4	12.0	16.9 ± 2.1	156.4	20.9 ± 2.4
Sarcoma 180	—	5 × 5	—	12.3 ± 1.1	—	29.3 ± 3.1
	Aminopterin	5 × 5	0.23	16.1 ± 2.4	31.0	25.1 ± 2.9
	10-Deaza-aminopterin	5 × 2	9.0	30.1 ± 4.1	145.5	27.2 ± 3.5
	Methotrexate	5 × 5	12.0	20.1 ± 2.9	63.2	26.0 ± 4.3
Ehrlich ascites	—	5 × 3	—	18.2 ± 1.8	—	29.2 ± 1.3
	Aminopterin	5 × 3	0.23	20.3 ± 3.0	11.5	29.5 ± 2.2
	10-Deaza-aminopterin	5 × 2	9.0	31.2 ± 4.6	71.4	28.8 ± 3.1
	Methotrexate	5 × 3	12.0	20.9 ± 3.5	32.0	29.1 ± 4.0

*No. of mice in each experiment × No. of experiments.
†Given as a single sc injection.
‡Initial weight = 20 g.

TABLE 4.—Effects of methotrexate and 10-deaza-aminopterin against solid forms of Sarcoma 180, Ehrlich ascites, and Taper liver tumors

Tumor	Drug	Dose* (mg/kg)	ATV ± SD† (mg)	%‡	Toxic deaths§
Sarcoma 180	—	—	149 ± 26	100	0/10
	Methotrexate	6	61 ± 8	41	0/10
	10-Deaza-aminopterin	6	18 ± 5	12	1/10
Taper liver	—	—	265 ± 21	100	0/10
	Methotrexate	6	81 ± 12	31	2/10
	10-Deaza-aminopterin	6	42 ± 6	16	2/10
Ehrlich ascites	—	—	99 ± 19	100	0/10
	Methotrexate	6	29 ± 5	30	1/10
	10-Deaza-aminopterin	6	20 ± 6	20	2/10

*Drug given as a single sc dose q1d starting 1 day after tumor transplantation for a total of 5 doses.
†Average of 2 experiments (5 mice/group). ATV = average tumor volume in mg recorded on Day 7.
‡% = $T(V_t-V_o)/C(V_t-V_o) \times 100$. $V(mm^3) = 4/3\ \pi r^3$ (diameter = 2 mm initially and 8 ± 2 mm on Day 7).
§No. of deaths/No. of mice treated.

focusing on membrane transport differences among analogs.

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Clinical Trial of Rubidazone in Solid Tumors and Malignant Lymphomas[1]

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SUMMARY

Rubidazone, a semisynthetic daunorubicin derivative, was administered to 33 patients with advanced solid tumors or malignant lymphomas. The doses used were 200, 150, or 100 mg/m^2 repeated at 3-week intervals. The antitumor effect was very modest. Partial remission was obtained in only one patient and stable disease was observed for periods of 4–18 weeks in seven additional patients. The major toxic effects were gastrointestinal and myelosuppressive. In two patients, cardiac toxic effects were noted and one patient had an anaphylactic-like reaction. No clinical cross-resistance seemed to exist between rubidazone and adriamycin.

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In the search for new potent analogs of the anthracycline antibiotics, rubidazone was introduced in clinical practice in 1972 (1). Chemically, rubidazone is a semisynthetic derivative of daunorubicin in which a benzoyl-hydrazone group is substituted at position 13 in the aglycone component. In Ehrlich ascites tumor (2), Sarcoma 180 (3), and L1210 leukemia (3,4), the therapeutic effect of rubidazone has proved to be similar to that of daunorubicin and adriamycin, whereas in B16 melanoma, its effect has been better compared to daunorubicin (4). In man, although rubidazone has been shown to exert a pronounced activity against acute leukemia (5), few data are available on the effect of the compound in patients with solid tumors and malignant lymphomas (6,7), for which reason the present study was initiated.

MATERIALS AND METHODS

Only patients with biopsy-proven malignant tumors in an advanced stage of their disease and with measurable lesions were included in the study. Most patients were clinically resistant to conventional chemotherapy and/or radiotherapy. However, none of the patients had previously received daunorubicin or adriamycin. No chemotherapy or radiotherapy had been given for 2 weeks (for BCNU and CCNU, 6 weeks) before the start of rubidazone therapy. Chlorpromazine was given as an antiemetic in five patients; barbiturates or antihistamines were not used. Each patient had a complete physical examination, and laboratory tests included a cbc count, determination of hemoglobin, serum creatinine, serum calcium, uric acid, bilirubin, alkaline phosphatase, SGOT, and urinalysis. Roentgenographic studies consisted of chest roentgenograms, and lymphangiograms were performed in all patients with malignant lymphoma. Electrocardiograms (ECGs) were obtained routinely once a week. The hematologic requirements for inclusion in the study consisted of a wbc count $\geq 4000/\mu l$ and platelet count $\geq 100{,}000/\mu l$, unless lower counts were disease-related. In addition, serum bilirubin had to be < 1.2 mg/100 ml and serum creatinine < 2.5 mg/100 ml.

Rubidazone was kindly supplied by the Rhone-Poulenc Laboratory (Paris, France). It was available as a lyophilized red powder in the form of monohydrochloride in vials containing 50 mg (calculated as base). The contents of the vial were dissolved in 4 ml of a solution containing 1.488 g of glycine and 0.0145 g of sodium glycinate in 100 ml of distilled water. The drug was administered over 2–3 minutes through the tubing of a freely running iv infusion (solution with isotonic saline or glucose).

Three dose levels were used: 200, 150, and 100 mg/

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m^2 repeated every 3 weeks. In the first patients a dose of 200 mg/m^2 every 3 weeks was used. Because this dose elicited significant toxicity, two lower dose levels, 150 and 100 mg/m^2, were studied. No other cytostatic drugs were administered concomitantly other than prednisone given in a low dose (< 30 mg/day) in a few patients. If tumor progression occurred, treatment was discontinued after the first or second course of therapy. In the case of no change or response, the therapy was continued until progression was seen. Tumor size was determined as the sum of the product of two diameters of measured lesions. Standard criteria were used for evaluation of response with complete remission (CR) indicating complete disappearance of all measurable disease, and partial remission (PR) indicating a reduction in tumor by > 50%. No change indicated 0–50% regression of measurable disease lasting at least 4 weeks, without the appearance of new lesions. Tumor progression was defined as an increase of at least 50% in the size of measured lesions or the appearance of significant new lesions.

RESULTS

Toxicity

The most frequent toxic effects observed were gastrointestinal and hematologic. Of the 33 patients, 14 complained of vomiting, nausea, or anorexia. No correlation was observed between dose of rubidazone and vomiting or nausea. Anorexia usually started within 24 hours after the injection of rubidazone and persisted for 1–2 weeks. Table 1 shows the degree of hematologic toxicity. The depression was more pronounced for leukocytes than for hemoglobin and thrombocytes. With increasing doses of rubidazone, an increased hematologic toxic effect was observed. Thus, three of eight patients (37.5%) treated with 100 mg/m^2 had leukopenia of grades I–IV, as compared with ten of 15 (66%) receiving 150 mg/m^2 and three of three (100%) receiving 200 mg/m^2. One patient died with pancytopenia and one patient developed sepsis. The nadir of leukopenia and thrombocytopenia occurred between Days 7 and 14. At 100 and 150 mg/m^2, hematologic recovery was usually observed between the 14th and 21st day. However, in one patient receiving 150 mg/m^2, recovery lasted for 6 weeks.

ECG changes following rubidazone therapy were seen in two patients. One patient (No. 28), after receiving a total cumulative dose of 720 mg/m^2, developed transient multifocal ventricular beats and flattening of T waves. The second patient (No. 8) had previously received radiotherapy to the abdomen (abdominal bath) and chemotherapy consisting of prednisone, vincristine, and cyclophosphamide. Following only one injection of rubidazone (150 mg/m^2), this patient developed tachycardia and low-voltage and supraventricular ectopic beats. He died from pulmonary edema 18 days after receiving the rubidazone injection. An autopsy showed moderate fibrosis of the myocardium, especially of the septum. The pericardium was not affected and no thrombosis or coronary occlusion was observed, but considerable arteriosclerosis was seen.

In one patient, an anaphylactic-like reaction with hypotension and unconsciousness was observed immediately after the injection of the first course of rubidazone. The reaction was controlled with iv adrenaline and the patient recovered completely within 30 minutes.

Five patients complained of muscle pain or a burning feeling during the first few days after the injection. Mucositis was rare and mild, and only one patient (receiving a total dose of 1050 mg/m^2) developed alopecia.

Therapeutic Effect

The 33 patients included in the trial had the following diseases: non-Hodgkin's lymphomas, nine; bronchogenic carcinoma, eight; cancer of the breast, seven; cancer of the uterus, two; cancer of the

TABLE 1.—Hematologic toxicity of rubidazone treatment

Dose (mg/m^2)	Total No. of patients	No. of patients evaluable for toxicity	No. of patients with—*											
			Anemia				Leukopenia				Thrombocytopenia			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
100	9	8	1				2		1		2			
150	19	15	2	1			3	4	3		2	1		
200	5	3		1	1		1	1		1				1

*Grade of severity: *anemia*—I = 6.2–7.4 mmols/liter (10–12 g/100 ml), II = 5.9–6.2 mmols/liter (9–10.0 g/100 ml), III = 4.3–5.9 mmols/liter (7–9.0 g/100 ml), IV = ≤ 4.3 mmols/liter (≤ 7.0 g/100 ml); *leukopenia*—I = 3000–4000 cells/μl, II = 2000–3000 cells/μl, III = 1000–2000 cells/μl, IV = < 1000 cells/μl; *thrombocytopenia*—I = 100,000–150,000 platelets/μl, II = 50,000–100,000 platelets/μl, III = 20,000–50,000 platelets/μl, IV = < 20,000 platelets/μl.

TABLE 2.—Characteristics of patients treated with rubidazone

Patient No.	Age/sex	Diagnosis*	Duration (mos)	Prior treatment†	Extent of disease	Rubidazone treatment: Dose (mg/m²)	No. of courses	Total dose (mg/m²)	Objective response‡	Response duration (wks)	Survival (wks)
1	63/F	Lymphoma, NLWD	86	S,C	Stage III	150	1	150	F		30+
2	40/F	Lymphoma, NLPD	56	C	Stage III	100	2	200	F		33
3	71/F	Lymphoma, DU	5	C	Stage IV	100	2	200	F		22+
4	41/F	Lymphoma, DLPD	47	C	Stage IV	150	1	150	F		28
5	66/M	Lymphoma, DM	6	C	Stage IV	150	1	150	F		39
6	56/M	Lymphoma, DU	3	C	Stage IV	150	3	435	PR	5	14
7	46/F	Lymphoma, DH	4	C	Stage IV	150	1	150	F		2
8	56/M	Lymphoma, DH	8	S,R,C	Stage IV	150	1	150	F		2
9	77/F	Lymphoma, DH	10	C	Stage IV	150	2	300	F		14+
10	58/F	Carcinoma of the breast	40	S,R,C	Skin and axillary nodes	150	3	250	F		8
11	61/F	Carcinoma of the breast	46	S,R,C	Skin, suprarenal glands, and supraclavicular nodes	150	1	150	F		18
12	37/F	Carcinoma of the breast	46	S,R,C	Lung, pleural effusion, and bone	200	7	770	NC	14	54+
13	44/F	Carcinoma of the breast	53	S,R,C	Skin and axillary nodes	150	4	575	NC	6	48+
14	52/F	Carcinoma of the breast	54	S,R,C	Skin, hepar, and axillary nodes	100	2	150	F		30
15	45/F	Carcinoma of the breast	55	S,R,C	Lung, skin, and axillary and supraclavicular nodes	150	6	850	NC	15	32+
16	60/F	Carcinoma of the breast	78	S,R,C	Skin and bone	100	3	300	F		36+
17	65/M	Carcinoma of the lung (squamous cell)	7	R	Lung, hilar mass, and supraclavicular nodes	150	1	150	F		1
18	59/M	Carcinoma of the lung (squamous cell)	14	R,C	Lung and costae	100	3	215	F		9
19	36/F	Carcinoma of the lung (adenocarcinoma)	9	S,R,C	Lung, breasts, and skin	100	2	200	F		15
20	60/M	Carcinoma of the lung (adenocarcinoma)	12	S,R	Lung, mediastinal mass, and cervical nodes	150	2		NC	4	6
21	45/F	Carcinoma of the lung (small cell anaplastic)	3		Lung, bone, chest wall, and supraventricular and axillary nodes	200	1	200	F		1
22	70/M	Carcinoma of the lung (small cell anaplastic)	4	C	Lung, skin, hepar, and axillary nodes	150	1	150	F		4
23	73/F	Carcinoma of the lung (small cell anaplastic)	9	R,C	Lung, hilar mass, and supraclavicular nodes	200	1	200	F		2
24	68/M	Carcinoma of the lung (small cell anaplastic)	12	R,C	Lung and axillary nodes	150	1	150	F		5

TABLE 2.—Characteristics of patients treated with rubidazone—Continued

Patient No.	Age/sex	Diagnosis*	Duration (mos)	Prior treatment†	Extent of disease	Rubidazone treatment: Dose (mg/m²)	Rubidazone treatment: No. of courses	Rubidazone treatment: Total dose (mg/m²)	Response: Objective response‡	Response: duration (wks)	Survival (wks)
25	63/F	Cancer of the ovary (sarcoma)	2	S,R	Abdominal mass and ascites	200	1	200	F		7
26	49/F	Cancer of the ovary (adenocarcinoma)	22	S,R,C	Pelvic mass, hepar, and ascites	100	2	100	F		1
27	63/F	Cancer of the uterus (mixed tumor)	13	R	Lung, vagina, and bone	150	7	1050	NC	16	58+
28	51/F	Cancer of the uterus (endometrial sarcoma)	72	S	Vagina, and pelvic and intestinal mass	150	6	720	NC	18	54+
29	68/M	Carcinoma of the cardia of esophagus	114	S,C	Lung and pleura	150	1	150	F		3
30	74/F	Primary cancer of the liver	1		Hepar	100	2	200	F		4
31	63/F	Poorly differentiated mesenchymal tumor	5	S	Lung and inguinal nodes	100	2	200	F		5
32	39/F	Carcinoma of the hard palate	140	S,R,C	Orbita, basis cranii, and lung	150	5	750	NC	12	35
33	61/F	Nonchromaffin paraganglioma	2	S,R,C	Hilar mass and supraventricular nodes	200	2	335	F		24

*N = nodular; D = diffuse; L = lymphocytic; M = mixed; H = histiocytic; U = undifferentiated; WD = well-differentiated; PD = poorly differentiated.
†C = chemotherapy; R = radiation therapy; S = surgery.
‡NC = no change; F = failure.

ovary, two; cancer of the esophagus, one; cancer of the liver, one; carcinoma of the hard palate, one; nonchromaffin paraganglioma, one; and a mesenchymal tumor, one.

The objective results are listed in table 2. Among the 33 patients studied, no CRs were observed. A PR lasting 5 weeks was observed in one patient with malignant lymphoma. In seven patients, the tumor remained unchanged for 5–18 weeks.

In six patients in whom tumor progression was observed during the rubidazone therapy, adriamycin was subsequently administered in doses of 60–75 mg/m^2 every 3 weeks or in a weekly regimen (8). In one patient (No. 3), CR was obtained even after the second dose of adriamycin was given in a dose of 60 mg/m^2 every 3 weeks. In this patient, adriamycin therapy was started 4 weeks after cessation of rubidazone and continued for 20 weeks, whereupon relapse in the CNS was observed. Neither the rubidazone treatment nor the adriamycin treatment elicited significant leukopenia or thrombocytopenia. The other five patients (Nos. 4–6, 9, and 14) showed stable disease or a tumor regression lasting 4–10 weeks which did not qualify for PR. Rubidazone treatment caused leukopenia in three of the five patients (grades I, II, and III respectively). Adriamycin was started 3–12 weeks after cessation of rubidazone therapy and a hematologic toxic effect (leukopenia, grade I) was noted in only one patient.

DISCUSSION

The results of the present study indicate that the antitumor activity of rubidazone in this intermittent dose schedule was only modest in the solid tumors and malignant lymphomas we studied. The conclusion is not changed even if five patients with a short life expectancy (survival < 4 weeks after the start of rubidazone treatment) are excluded from the study. With a similar schedule, Benjamin et al observed seven patients with stable disease among 19 patients with solid tumors (6). In another study (7), 21 patients with solid tumors were treated with rubidazone, 1–2 mg/kg over 3 days with an interval of at least 3 days between courses, resulting in a PR in one patient. In view of the promising results obtained with rubidazone treatment of myeloblastic and lymphoblastic leukemia (5), these findings suggest that rubidazone is a narrow-spectrum antineoplastic compound like daunorubicin (9).

In Ehrlich ascites tumor, acquired reciprocal cross-resistance has been demonstrated between the anthracycline antibiotics rubidazone, daunorubicin, and adriamycin (2). The fact that six patients clinically refractory to rubidazone subsequently responded to adriamycin may reflect a difference in the pattern of primary resistance between the two anthracyclines. This difference may be due to the differences in the metabolism (10), the cellular drug uptake (11), or the effect on nucleic acid synthesis (12) described.

The therapy was associated with significant toxicity to the bone marrow. The interval of 3 weeks between the injections seems adequate. In almost all instances, leukocyte and platelet counts had returned to normal levels after this interval. Doses of 100 and 150 mg/m^2 were tolerated whereas 200 mg/m^2 resulted in a high frequency of severe hematologic toxicity.

In studies in rats, rubidazone seemed to be less cardiotoxic compared to daunorubicin and adriamycin (13). In our study, cardiac symptoms were noted in two patients. One patient may reflect nonspecific ECG changes; according to Lefrak et al (14) this phenomenon is not related to the serious cumulative cardiomyopathy. In the other patient, the etiology is difficult to evaluate. Thus, considerable arteriosclerosis in the coronary arteries was noted; furthermore, this patient had received cyclophosphamide which may be a risk factor for anthracycline-induced cardiomyopathy (15). In another study, six of 21 patients had ECG changes after a total dose of 150–1750 mg (7); however, no evidence was presented indicating the existence of chronic cardiomyopathy. Cardiac toxic effects have also been observed in patients treated with small total doses of daunorubicin (16–18) as well as adriamycin (19–21). On the other hand, in large series of adults, the incidence of cardiomyopathy has been calculated as very low at a cumulative dose of these drugs of < 550 mg/m^2 (14,22,23).

In conclusion, rubidazone in the doses and regimens used in this and other published series (6,7) seems to have only modest antitumor activity in solid tumors and malignant lymphomas.

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Combination Chemoimmunotherapy for Extensive Non-Oat Cell Lung Cancer[1,2]

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SUMMARY

In a prospective randomized trial, sc *Corynebacterium parvum (C. parvum)* immunotherapy did not significantly affect the response and survival of 49 non-oat cell lung cancer patients receiving isophosphamide and adriamycin chemotherapy. Remissions (tumor regression > 50%) were seen in five of 23 patients receiving an intensive *C. parvum* schedule and in three of 26 patients receiving a nonintensive *C. parvum* schedule (22% versus 12%). Median survival was 20 weeks for patients given intensive *C. parvum* and 23 weeks for patients given nonintensive *C. parvum*. This study did demonstrate the importance of pretherapy immunocompetence, performance status, and weight loss as predictors for survival. Weight loss was the most significant prognostic factor. Performance status was closely associated with weight loss but skin reactivity to dermatophytin predicted independently and was the second most important prognostic characteristic.

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Adriamycin appears to be one of the most active single agents in non-oat cell lung cancer patients (1). Isophosphamide also has shown promising activity in this patient group (2). Sc administered *Corynebacterium parvum (C. parvum)* has been reported to prolong survival and to reduce chemotherapy-induced myelosuppressive toxicity when added to chemotherapy in non-oat cell lung cancer patients (3). Also, *C. parvum* administered iv alone has resulted in significant tumor regression even in patients with advanced disease (4). It was decided to investigate two sc schedules of *C. parvum* added to isophosphamide and adriamycin chemotherapy with the hope that the intensive *C. parvum* schedule would more closely simulate the iv route.

PATIENTS AND METHODS

Consecutive patients with inoperable histologically proven non-oat cell bronchogenic carcinoma who had not previously received therapy with any of the three agents were entered in the study. Patients were randomized to one of two *C. parvum* schedules. Patients entered in the study were stratified according to histology, prior radiotherapy, weight loss, performance status, and sex. A signed informed consent was obtained prior to therapy according to institutional policy.

The chemotherapy was administered in 3-week cycles as follows: isophosphamide (1000 mg/m² in 200 ml of 5% dextrose solution) was given over a 2-hour period daily × 5 days. To reduce urinary complications, patients were encouraged to drink 3 quarts of fluid daily and to void frequently over this period. Adriamycin (60 mg/m² in 50 ml of 5% dextrose solution) was given by rapid iv infusion on

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Day 1 of each course of isophosphamide. Therapy with adriamycin was discontinued after a total cumulative dose of 450 mg/m². *C. parvum* immunotherapy was given sc at a dose of 2 mg/m² by one of the following schedules: (*a*) an intensive schedule consisting of doses administered daily for 10 consecutive days prior to the first course of chemotherapy, on Days 7–16 of courses 1 and 2, and on Days 7, 12, and 17 of subsequent courses of chemotherapy; or (*b*) a nonintensive schedule consisting of doses on Days 7, 12, and 17 of each course of chemotherapy.

Evaluation of measurable disease by full physical examination and chest roentgenogram was documented before each course for the first three courses, and then at 6–12-week intervals. A remission was defined as ≥ 50% decrease in the sum of the products of two perpendicular diameters of all measured lesions without simultaneous increase in the size of any lesion or the appearance of any new lesion; improvement was defined as measured regression < 50%; stable disease was defined as no measured change or an increase of < 25% for ≥ 12 weeks; and progressive disease was defined as an increase of ≥ 25% in the size of any measured lesion or the development of new lesions. To assess toxicity, a urinalysis was done on each day of isophosphamide infusion to document hematuria. Also, cbc, differential, and platelet counts were done at least twice weekly. Delayed hypersensitivity skin tests to recall antigens dermatophytin, Varidase, *Candida*, and mumps were placed intradermally between 48 and 72 hours prior to therapy according to the technique of Hersh et al (5). Induration was measured at 24 and 48 hours. The data recorded were the average of two right-angle measurements of the induration at the time it was the largest. For each antigen, the level of induration considered positive was that which had been determined previously by multivariate stepwise logistic regression analysis in lung cancer patients (5). The Karnofsky scale was used to determine performance status (6). The percentage scale was further divided into three classes as follows: 70%–100%, class A; 50%–70%, class B; and 10%–40%, class C. Survival calculations for patients entering this study were determined from the point of initiation of treatment by the method of Kaplan and Meier (7) for censored and uncensored data. The differences between survival curves were evaluated by the two-tailed generalized Wilcoxon test according to Gehan (8). The relative prognostic significance of several patient characteristics was determined by multivariate regression methods as proposed by Cox (9) and later used by Lanzotti et al (10) in lung cancer patients.

TABLE 1.—Characteristics of patients entered in each *C. parvum* immunotherapy schedule

Characteristic	*C. parvum* therapy schedule	
	Intensive	Nonintensive
No. of patients	23	26
Men	18	18
Women	5	8
Median age in yrs (range)	55 (28–66)	56 (36–68)
Prior radiotherapy	15	15
Histologic type		
Squamous cell carcinoma	12	13
Adenocarcinoma and large cell carcinoma	11	13
Performance status (Karnofsky)		
Class A	12	11
Class B and C	13	13
Weight loss prior to therapy		
< 6%	13	14
≥ 6%	10	12

RESULTS

Patient Characteristics

Forty-nine patients with extensive disease as defined by the Veterans' Administration Lung Cancer Study Group criteria (11,12) were entered in the study. Two patients died of respiratory failure secondary to tumor progression within 2 weeks of entering the study (one for each schedule), but have been included in the study analysis.

Table 1 shows the distribution of patient characteristics between the two *C. parvum* groups. The prognostic factors of weight loss prior to therapy and performance status were evenly distributed. Only one patient had received prior chemotherapy (intensive group).

Response and Survival

Table 2 shows the response rates of the two *C. parvum* groups. No significant difference was seen between the two schedules both overall or for each tumor histology type. Remissions (tumor regression > 50%) were seen in five patients receiving intensive *C. parvum* and in three patients receiving nonintensive *C. parvum* (22% versus 12%). However, 13 of 17 patients with improvement or stable disease had disease which was not measurable for remission. This was generally because prior radiotherapy did not permit the accurate measurement

TABLE 2.—Response related to *C. parvum* immunotherapy schedule

Response	*C. parvum* therapy schedule: Intensive (No. of patients)	*C. parvum* therapy schedule: Nonintensive (No. of patients)	*P* value
Squamous cell carcinoma			
Remission	4 (4 of 12 patients, 33%)	1 (1 of 13 patients, 8%)	0.27
Improvement	2	2	
Stable disease	3	4	
Progression	3	6	
Adenocarcinoma and large cell carcinoma			
Remission	1 (1 of 11 patients, 9%)	2 (2 of 13 patients, 15%)	1.0
Improvement	0	2	
Stable disease	3	1	
Progression	7	8	
Overall			
Remission	5 (5 of 23 patients, 22%)	3 (3 of 26 patients, 12%)	0.32
Improvement	2	4	
Stable disease	6	5	
Progression	10	14	

of disease regression. There was no significant difference in survival between the two schedules of *C. parvum*. The median survival for the intensive *C. parvum* group was 22 weeks and for the nonintensive group was 20 weeks. Increased survival was significantly related to pretreatment performance status, weight loss, and skin test results, but not to histology (table 3). A multivariate regression analysis of the relative prognostic significance of these characteristics showed that weight loss was the most significant. Performance status was closely associated with weight loss but skin reactivity to dermatophytin predicted independently and was thus the second most important characteristic. The addition of performance status or the other skin test reactivities did not increase the prognostic significance over that determined by weight loss and dermatophytin skin testing.

Toxicity

Myelosuppression, mainly leukopenia, was the most common toxic effect. Data in table 4 were determined from 125 evaluable courses of therapy. There was no evidence of cumulative myelosuppression in 11 patients who had five or more consecutive courses of therapy analyzed. The schedule of immunotherapy did not appear to influence the degree of myelosuppression. Ten percent of the patients required hospitalization at some time during their therapy for presumed or proven infection related to leukopenia. There were two episodes of pneumonia, one of pneumonia and septicemia, and three of fever of unknown origin. There were no drug-related deaths.

Fifty-two percent of the patients experienced nausea and vomiting and 10% had major gastrointestinal side effects requiring parenteral rehydration. Hematuria not usually associated with dysuria was documented in 50% of the patients. Macroscopic hematuria was noted in 21% of the patients and microscopic (> 5 rbcs/high-powered field) was noted in an additional 29%. Eight percent of the patients had major hematuria requiring interruption of isophosphamide therapy. Local soreness at the *C. parvum* sc injection sites was reported in 30% of the patients. Fever related to *C. parvum* administration was documented in 8% of the patients and a flu-like syndrome was noted in 2%. Four percent of the patients had major local reactions to *C. parvum* requiring modification of further therapy by dosage reduction.

DISCUSSION

In a prospective randomized trial, sc *C. parvum* immunotherapy, when added to chemotherapy, did not affect the response or survival of patients with metastatic non-oat cell carcinoma. Also, the overall efficacy of this treatment does not appear to represent a significant advance when compared to other reported regimens. Our previous experience in similarly selected patients with squamous cell lung

TABLE 3.—Factors affecting survival

Factor	No. of patients	Median survival (wks)	*P* value
C. parvum schedule			
Intensive	23	22	0.63
Nonintensive	26	20	
Histology			
Squamous cell carcinoma	25	22.0	0.69
Adenocarcinoma and large cell carcinoma	24	23.5	
Weight loss			
< 6%	25	Not reached	0.002
≥ 6%	24	14.3	
Performance status			
Class A	23	38.9	0.002
Class B and C	26	15.7	
Skin tests			
Dermatophytin > 10 mm	21	Not reached	0.002
Dermatophytin < 10 mm	22	14.0	
Candida > 10 mm	32	24.0	0.01
Candida < 10 mm	12	12.0	
Varidase > 20 mm	7	Not reached	0.05
Varidase < 20 mm	37	20.6	
Mumps > 5 mm	25	25.0	
Mumps < 5 mm	19	19.0	0.22

TABLE 4.—Myelosuppressive toxicity in relation to *C. parvum* schedule

	Lowest recorded count × 10^3/mm^3		
	Overall	Intensive *C. parvum*	Nonintensive *C. parvum*
Wbc count			
Median	1.9	2.0	1.8
Range	0.6–9.5	0.7–6.0	0.6–9.5
Day	12	12	12
Absolute neutrophil count			
Median	1.1	1.4	1.1
Range	0.01–8.4	0.02–5.6	0.01–8.4
Day	12	14	12
Platelet count			
Median	260	268	220
Range	43–900	43–900	33–821
Day	12	11	12

cancer was with bleomycin, adriamycin, CCNU, vincristine, and mechlorethamine (BACON) chemotherapy where the median survival for patients with extensive disease was 16 weeks (13). With the exception of a study reported by Chahinian and others (14) where the predicted median survival promises to be higher, most other recently reported chemotherapy combinations (15–18) have resulted in patient survivals similar to that noted in our study. This is disappointing considering the encouraging data on isophosphamide as a single agent (2). *C. parvum* immunotherapy also does not seem to have significantly added to the efficacy of the chemotherapy nor does it appear to have protected patients from the toxicities of adriamycin and isophosphamide. However, the value of *C. parvum* in this tumor group should not be considered negative on this basis since the route and schedule of administration may be suboptimal. Also, it may be that there is insufficient difference between the intensities of

each *C. parvum* schedule to show a comparative therapeutic benefit both in response and protection against chemotherapy toxicity.

This study further demonstrates the importance of pretherapy weight loss, performance status, and immunocompetence as prognostic factors. Our findings that weight loss and skin reactivity to dermatophytin were the most important independently predicting characteristics have not previously been described. The documentation of these characteristics for all lung cancer patients entering studies would allow a better comparison of patient populations and thus a more accurate assessment of the relative efficacy of different therapies.

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Effects of 5-Fluorouracil on Human Colon Carcinoma and Solid Rat Walker 256 Carcinosarcoma: Evaluation as In Vitro Predictors of Clinical Response [1,2]

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SUMMARY

Several biochemical effects of 5-fluorouracil (5-FU) including inhibition of the incorporation of ^{3}H-deoxyuridine (^{3}H–UdR) into DNA, inhibition of ribosome formation, and formation of 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) were examined in samples of human colon carcinomas to determine if any of these drug effects might have predictive value as a reliable guide to 5-FU therapy. For comparison, the solid rat Walker 256 carcinosarcoma, which is only minimally responsive to 5-FU, was also studied. For each of the biochemical effects of 5-FU measured in the various samples of Walker 256 tumors, the responses were consistent and varied within a narrow range. In contrast, the formation of FdUMP from 5-FU and the degree of inhibition of the incorporation of ^{3}H-UdR into DNA by 5-FU were extremely variable in the population of human colon carcinomas examined. In all human tumors examined, 5-FU caused a reduction in the formation of ribosomes, but even in the absence of 5-FU, the total amount of ribosome synthesis was so low that it makes measurement difficult to quantitate. Based on our data, a study might be warranted to determine if there is a correlation between FdUMP formation and responsiveness of colon carcinoma to 5-FU therapy.

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One of the major problems in clinical cancer chemotherapy is the lack of a simple, rapid, and reliable technique for predicting the antitumor response prior to the initiation of chemotherapy. The development of an in vitro system to test drug sensitivity to anticancer drugs, for example, by using human solid tumor samples obtained at surgery, would allow for individualized therapy and would spare patients delays in instituting effective therapy and the ordeal of ineffective drug treatment involving further tumor growth and drug toxicity (1). This problem is particularly acute for the use of the antimetabolite 5-fluorouracil (5-FU) in the treatment of disseminated colon carcinoma because the response rate is only about 25% (2).

Two major drug effects of 5-FU have been consistently established. 5-FU in the form of its anabolite 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) inhibits the enzyme thymidylate synthetase and thereby blocks DNA synthesis. This is usually considered to be its major growth inhibitory effect (3). In addition, studies in various species have shown that 5-FU is extensively incorporated into messenger, ribosomal, and transfer RNA and also inhibits ribosomal RNA maturation (3,4). Studies in this laboratory with microorganisms have indicated that inhibition of ribosome synthesis by 5-FU correlates more closely with early growth inhibition than does blockade of DNA synthesis (5,6). Thus, although much is known about the mechanism of action of 5-

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FU, there is as yet no clear guide to a specific biochemical effect which can serve as a consistent predictor of the clinical response to 5-FU (7).

Other investigators have attempted to develop in vitro predictor systems for clinical response to 5-FU based, for example, on inhibition by 5-FU of incorporation of ^{14}C-formate into thymine DNA and incorporation of ^{3}H-thymidine (^{3}H-TdR) into DNA (8,9), or comparison of enzymatic pathways involved in 5-FU anabolism and of salvage pathways for TdR utilization (10). In a series of rodent tumors, anabolism of 5-FU to unspecified 5-FU nucleotides correlated with the enhanced response to 5-FU (11). At present, none of these approaches has proved to be a reliable predictor of clinical response to 5-FU. Techniques where human colon cancer cells are grown out in culture or transplanted to immune deficient mice may be subject to cloning out of unrepresentative tumor cell populations which presents a significant disadvantage in predicting tumor sensitivity to anticancer drugs.

Therefore, using freshly obtained samples of human colon carcinoma, we have examined two biochemical effects of 5-FU not previously examined, namely, inhibition of ribosomal synthesis and anabolism of 5-FU to FdUMP, to see if these assays might have potential value as a reliable guide to therapy. In addition, the effects of 5-FU on DNA synthesis, considered to be its primary site of action, were also examined. For comparative purposes, experiments were also carried out with the solid rat Walker 256 carcinoma, a rodent tumor which, like many human colon carcinomas, is only minimally responsive to 5-FU (12).

MATERIALS AND METHODS

Chemicals

^{3}H-[2,8]-adenosine (30 curies/mmol) and ^{3}H-[6]-deoxyuridine (^{3}H-UdR) (28 curies/mmol) were obtained from the New England Nuclear Corp (Boston, Mass). 5-FU was obtained as a gift from Hoffmann-La Roche Inc (Nutley, NJ). FdUMP and 2-deoxyuridine-5′-monophosphate (dUMP) were obtained from Sigma Chemical Co (St. Louis, Mo). Thymidylate synthetase, prepared from dichloromethotrexate-resistant *Lactobacillus casei* (*L. casei*) by the method of Crusberg et al (13), was obtained from New England Enzyme Center, Tufts University Medical School (Boston, Mass). Eagle's minimal essential medium was obtained from Industrial Biological Laboratories (Rockville, Md).

Animals and Tumor Preparations

The Walker 256 carcinosarcoma in the ascites form was maintained in male rats weighing 50–60 g, obtained from Sprague-Dawley Laboratories (Madison, Wis). Animals had free access to standard Purina laboratory chow and tap water. Ascites cells, 2×10^{6}, were injected sc into the right thigh of male Sprague-Dawley rats weighing 90–100 g. Seven days later, the animals were killed and the tumors (weighing 3–5 g) were removed. Slices (approximately 200 mg each) were prepared in the cold with a Stadie-Riggs hand microtome and placed in Eagle's medium containing 10% fetal calf serum for incubation at 37°C under 95% O_2–5% CO_2 in a Dubnoff type shaker.

Human tumor specimens were obtained at the time of surgery from patients with colon carcinomas and malignancy was verified by histologic examination. Immediately following excision, a sample for biochemical studies was placed in a beaker of ice. The tumor was dissected free of normal and necrotic tissue, and slices (approximately 200 mg each) were prepared as with the Walker 256 carcinoma. On the average, human tumor samples were processed within 20–30 minutes after excision.

Effect of 5-FU on DNA Synthesis

Approximately 0.6 g of tumor tissue in 5 ml of Eagle's medium containing 10% fetal calf serum was added to each flask. 5-FU, in 0.1 ml of 1% Na_2CO_3, was added to experimental flasks while 0.1 ml of 1% Na_2CO_3 was added to control flasks. Slices were preincubated for 1 hour with 5-FU at various concentrations followed by the addition of 1.0 ml of Eagle's medium containing ^{3}H–UdR (10 microcuries for Walker 256 carcinoma and 50 microcuries for human colon carcinoma) and a mixture of deoxyadenosine, deoxyguanosine, and deoxycytidine (0.5 μmol of each), and incubation continued for an additional hour. During this time, the incorporation of ^{3}H–UdR into DNA was found to be linear. The incubation was terminated by aspirating the Eagle's medium. The slices were washed with two 5-ml portions of cold Eagle's medium and with cold 15% trichloroacetic acid (TCA); they were then homogenized in the cold and centrifuged, and the supernatant was decanted. The precipitate was washed three times with cold 10% TCA, defatted with ethanol and ether, incubated at 37°C for 1 hour in 0.5 N KOH to hydrolyze RNA, and acidified with 50% TCA; the precipitate was washed two times with cold 10% TCA and then extracted two times with 5% TCA at 90°C. Aliquots of the hot TCA extract were analyzed for labeled DNA pyrimidines in Bray's solution (14) in a Beckman 250-DPM liquid scintillation counter and DNA was determined colorimetrically by the diphenylamine method (15). All

flasks were run in duplicate and the results were calculated after subtraction of zero time controls. In a series of experiments, the incorporation of ^{3}H-UdR into the DNA of control samples of Walker 256 carcinoma varied between 11,700 and 40,100 dpm/mg of DNA while human colon carcinoma control samples varied between 5400 and 41,500 dpm/mg of DNA.

Effect of 5-FU on Ribosome Synthesis

Tumor slices (approximately 0.6 g), in 5 ml of Eagle's medium containing 10% fetal calf serum, were added to each of three control and three experimental flasks. Slices were preincubated for 1 hour with or without 5-FU followed by the addition of 1.0 ml of Eagle's medium containing ^{3}H-adenosine (50 microcuries for either the Walker 256 or human colon carcinoma) and a mixture of guanosine, cytosine, and uridine (0.5 mol each), and incubation was allowed to proceed for 4 more hours. The incubation was terminated by aspirating the Eagle's medium and washing the slices with two 3-ml portions of 10 mM tris-0.1 mM magnesium acetate-60 mM KCl buffer, pH 7.8 (TMK). Slices from each of the three control or experimental flasks were pooled, homogenized in the cold in 3 ml of TMK buffer, and centrifuged at 9500 rpm for 20 minutes in a Beckman J-21B centrifuge; the supernatant was made 0.5% with respect to sodium desoxycholate and dialyzed at 4°C for 18 hours against 1000 volumes of TMK buffer. An aliquot of the dialyzed extract was then applied onto 5 ml of a linear (10%–30%) sucrose-TMK gradient prepared by using a Buchler gradient machine, and the tubes were centrifuged at 39,000 rpm for 110 minutes in an SW 50L rotor. Gradients (three control and three experimental flasks) were analyzed using an ISCO model 183 automatic density-gradient fractionator (Instrument Specialities Co, Lincoln, Neb), equipped with an ISCO model 222 single-beam ultraviolet analyzer connected to and recorded by an ISCO model 170 servograph recorder. Fractions of three drops (equivalent to 0.15 ml) were collected in vials, 10 ml of Bray's solution (14) was added, and the radioactivity was counted in a Beckman DPM-250 liquid scintillation counter.

FdUMP Formation From 5-FU

Tumor slices, approximately 0.6 g/flask, in 5 ml of Eagle's medium containing 10% fetal calf serum, were incubated for 4 hours with 5-FU, at which time the incubation was terminated by aspirating the Eagle's medium. The slices were washed with two 5-ml portions of cold Eagle's medium and then tissue samples were extracted with 1 M acetic acid and the acid-soluble extracts were analyzed for FdUMP according to the enzymatic method of Myers et al (16,17). This assay, based on the stoichiometric inhibition of *L. casei* thymidylate synthetase by FdUMP, follows the increase in absorbance at 340 nm associated with the conversion of $N^{5,10}$-methylenetetrahydrofolate to dihydrofolate at 340 nm in the presence of enzyme, dUMP, and Mg^{++}. All incubations were carried out in duplicate and a zero time control was used to determine dUMP levels in tumor slices before the addition of 5-FU. This method measures only the acid-soluble unbound FdUMP and not the total cellular FdUMP. The latter consists of unbound FdUMP as well as FdUMP covalently bound to thymidylate synthetase (3,16,17).

RESULTS

Effect of 5-FU on the Incorporation of ^{3}H-UdR Into the DNA of Rat Walker 256 and Human Colon Carcinomas

Table 1 shows the effect of 5-FU on DNA synthesis, as measured by incorporation of ^{3}H-UdR into DNA, in slices of rat Walker 256 and colon carcinoma samples from a series of six patients. 5-FU at

TABLE 1.—Effects of 5-FU on the incorporation of ^{3}H-UdR into DNA of rat Walker 256 and human colon carcinomas

Sample	5-FU (μM)	Incorporation of ^{3}H-UdR into DNA (% of control)*
Walker 256	2	51.6 ± 2.8†,‡
Walker 256	8	30.7 ± 7.6‡,§
Walker 256	200	3.1 ± 1.06¶,**
Patient No.		
1	200	21.4
2	200	16.7
3	200	72.1
4	200	76.1
5	200	43.8
6	200	77.1

*Following incubation the tumor slices were washed, precipitated with cold 15% TCA, extracted, and analyzed for radioactivity and DNA as described in the Methods section. Drug effects are calculated as % of control of the incorporation of ^{3}H-UdR into DNA (dpm/mg) over a 1-hr incubation period after 1 hr preincubation with 5-FU.

†$P < 0.02$ compared to control by method of paired comparisons.

‡Mean ± SE of 5 experiments.

§$P < 0.025$ compared to control by method of paired comparisons.

¶$P < 0.005$ compared to control by method of paired comparisons.

**Mean ± SE of 4 experiments.

200 μM (25 μg/ml) is equivalent to the maximum plasma concentration attained after a single iv injection of a therapeutic dose of the drug (15 mg/kg) in man (8). As can be seen, 5-FU (200 μM) markedly and uniformly inhibited DNA synthesis in rat Walker 256 to < 5% of control. In contrast, in samples from six patients, inhibition by 5-FU of DNA synthesis varied from 23% to 83% of control with a mean of 51%. In this series of six patients, two were treated with 5-FU subsequent to surgery. Patient 2 had a transient antitumor response, while patient 1 was unresponsive to 5-FU. In both patients, the in vitro test indicated about 80% inhibition of ^{3}H-UdR incorporation into DNA.

Effect of 5-FU on Ribosome Synthesis in Rat Walker 256 and Human Colon Carcinomas

A typical density-gradient analysis obtained after incubation of rat Walker 256 slices with ^{3}H-adenosine to measure ribosome synthesis in the absence or presence of 5-FU (200 μM) is shown in figure 1a and b, respectively. As shown (fig 1a), the distribution of radioactivity from ^{3}H-adenosine parallels the absorbancy at 254 nm in the soluble RNA (fractions 0–11), ribosomal subunits (fractions 12–24), and ribosomes (fractions 25–34). In contrast (fig 1b), 5-FU at 200 μM depressed the incorporation of ^{3}H-adenosine into ribosomal subunits (fractions 12–24), ribosomes (fractions 25–34), and, to a lesser extent, soluble RNA (fractions 0–11). Qualitatively similar results are seen in density-gradient analyses obtained after incubation of human colon carcinoma slices with ^{3}H-adenosine in the absence or presence of 5-FU (fig 2a and b). Although not shown, the effect of 5-FU on ribosome synthesis in the Walker 256 tumor was comparable when ^{3}H-guanosine was used instead of ^{3}H-adenosine as the labeled precursor.

In order to quantify the relationship between concentration of 5-FU and inhibition of ribosome synthesis in rat Walker 256 carcinoma, we calculated the percentage of radioactivity from ^{3}H-adenosine in the total extract that had been associated with ribosomes and ribosomal subunits, ie, $\frac{\text{sum of cpm in fractions 12–34}}{\text{sum of cpm in fractions 0–34}} \times 100$ (fig 1a and b). Similar calculations were done for density-gradient analyses in corresponding fractions of human colon carcinoma samples.

As shown in table 2 for the Walker 256 carcinoma, 5-FU at 8 and 200 μM decreased the radioactivity (from ^{3}H-adenosine) in ribosomal fractions to 77% and 44% of the control, respectively, while 5-FU at 2 μM had no effect. In contrast, as shown in table 1, 5-FU at 2, 8, and 200 μM depressed DNA synthesis to 52%, 31%, and 3% of control values, respectively. This discrepancy indicates a marked increase in

TABLE 2.—Effect of 5-FU on ^{3}H-adenosine distribution in ribosomal fraction of rat Walker 256 and human colon carcinomas

Sample	5-FU (μM)	% ^{3}H-adenosine in ribosomal fraction	% of control*
Walker 256	—	54.0 ± 2.0	—
	2	53.5 ± 2.1†	99
Walker 256	—	47.2 ± 0.6	—
	8	36.6 ± 2.1†,‡	77
Walker 256	—	55.7 ± 1.2	—
	200	23.7 ± 1.7†,§	43
Patient No.			
7	—	23.4	—
	200	12.9	55
8	—	18.8	—
	200	10.6	56
9	—	19.9	—
	100	12.8	64
10	—	22.1	—
	100	11.9	54

* Drug effects are calculated from density-gradient analyses as % of control of radioactivity (cpm) from ^{3}H-adenosine in ribosomes and ribosomal subunits over the combined total of radioactivity in soluble RNA plus ribosomes and ribosomal subunits.

† Mean ± SE of 4 experiments.

‡ $P < 0.005$ compared to control by method of paired comparisons.

§ $P < 0.001$ compared to control by method of paired comparisons.

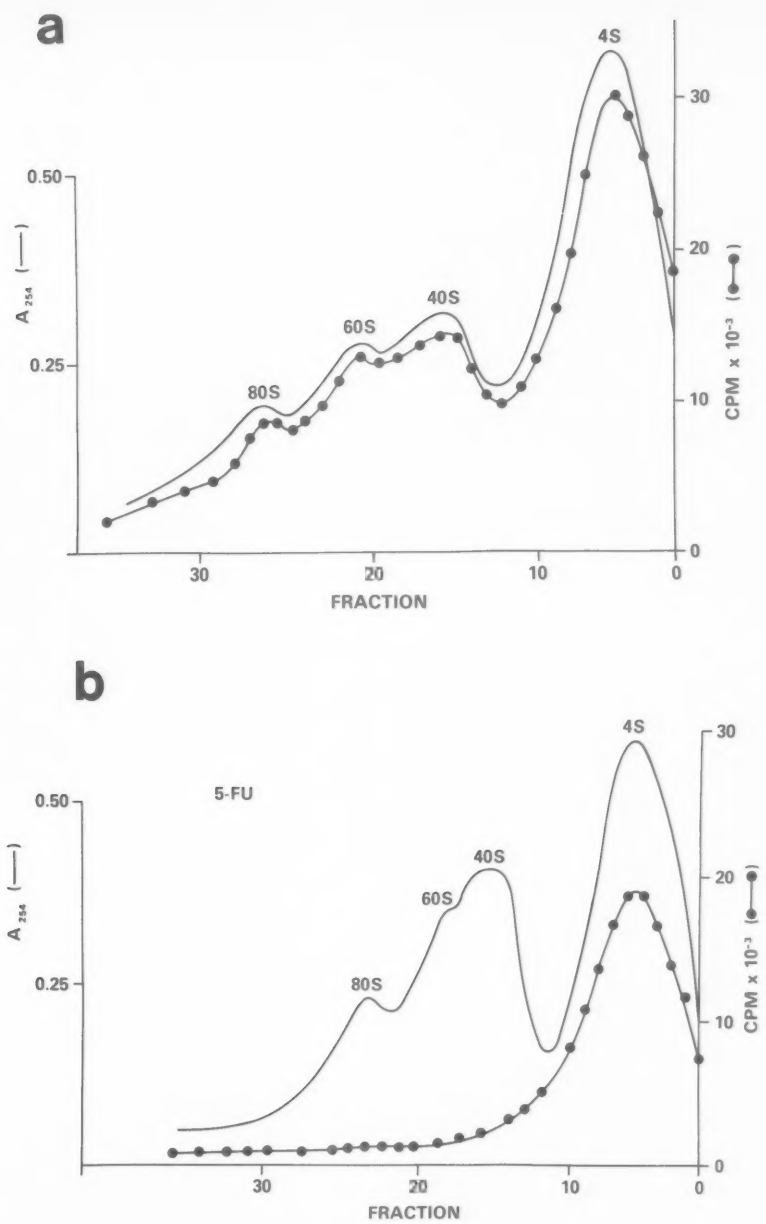

FIGURE 1 (a and b).—Sucrose density-gradient analysis of control and 200 μM 5-FU-treated Walker 256 carcinoma labeled with ^{3}H-[2,8]-adenosine. Following incubation, tumor slices were removed, washed, and homogenized, and the cell-free extract was dialyzed overnight against TMK buffer. The dialyzed extract was applied to 5 ml of 10%–30% sucrose-TMK gradient and centrifuged at 39,000 rpm for 110 mins. Gradients were analyzed for radioactivity and A_{254} as described in the Methods section. ●——● = cpm; —— = A_{254}.

sensitivity to inhibition by 5-FU of DNA versus that of ribosome synthesis in the rodent tumor.

As for human colon carcinoma, 5-FU at 100 and 200 μM decreased the incorporation of radioactivity from ^{3}H-adenosine into ribosomes to 54%–64% of the control (table 2). Since the radioactivity from ^{3}H-adenosine in control ribosomes averaged only 21% of the total radioactivity (as compared to 52% for the rat Walker 256 tumor ribosomes), the effect due to 5-FU was extremely difficult to quantify. In addition, following exposure to 5-FU, low but measurable amounts of radioactivity from ^{3}H-adenosine were still detectable in ribosomal fractions of both Walker 256 (fig 1b, fractions 15–34) and human colon (fig 2b, fractions 16–40) carcinoma tumors. Whether this radioactivity was due to incomplete inhibition of ribosome synthesis or is an artifactual background was not determined. However, if this background had been subtracted from control and 5-FU-treated samples, the inhibitory effect of 5-FU on

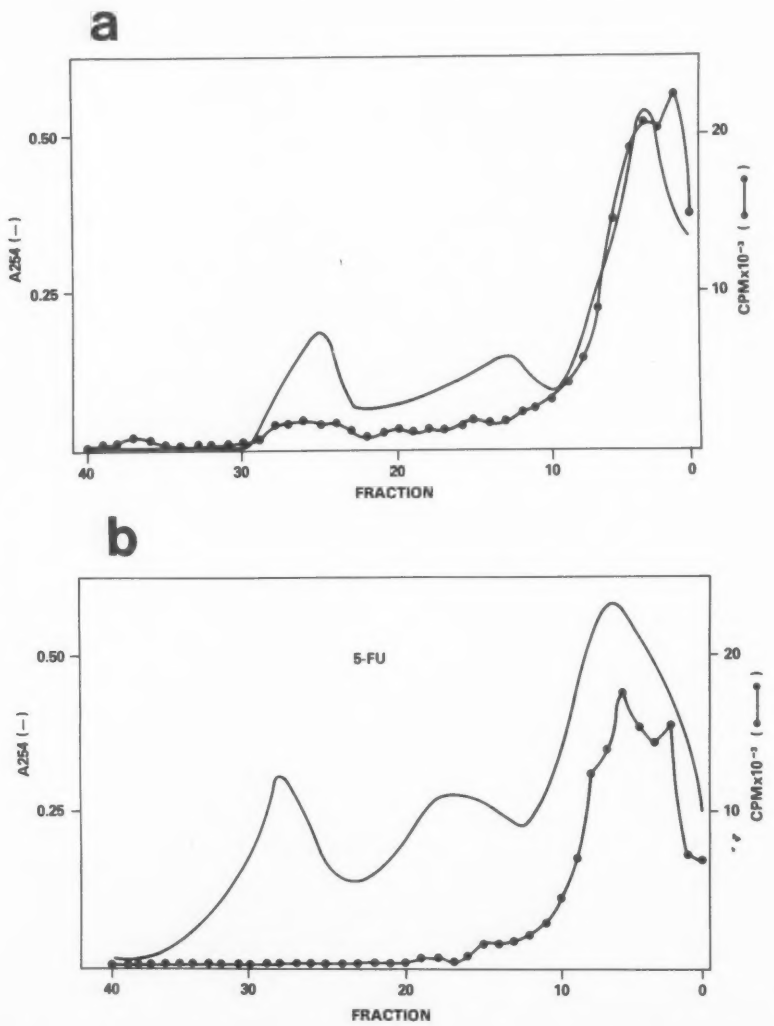

FIGURE 2 (a and b).—Sucrose density-gradient analysis of control and 200 μM 5-FU-treated human colon carcinoma labeled with ^{3}H-[2,8]-adenosine. Following incubation, tumor slices were removed, washed, and homogenized, and the cell-free extract was dialyzed overnight against TMK buffer. The dialyzed extract was applied to 5 ml of 10%–30% sucrose-TMK gradient and centrifuged at 39,000 rpm for 110 mins. Gradients were analyzed for radioactivity and A_{254} as described in the Methods section. ●——● = cpm; —— = A_{254}.

incorporation of ^{3}H-adenosine into ribosomes would have been greater for both tumors.

As shown in table 1, from a series of six patients, inhibition by 5-FU (200 μM) of the incorporation of ^{3}H–UdR into DNA of human colon carcinoma was so variable (17%–77% of control) that it was not possible (particularly with the limited number of samples studied) to conclude whether DNA or ribosome synthesis was more sensitive to inhibition by 5-FU.

Formation of FdUMP From 5-FU in Rat Walker 256 and Human Colon Carcinomas

An enzymatic assay for FdUMP has recently been developed (16,17). Using this method, we measured the formation from 5-FU of its active metabolite, FdUMP, in slices of rat Walker 256 and human colon carcinomas in order to determine if the formation of FdUMP might have predictive value clinically. In a series of tumor samples from 19 patients incubated with 5-FU, an average of 0.33 pmol of FdUMP/μg of DNA was formed (fig 3). In comparison, four Walker 256 tumors formed an average of 0.12 pmol of FdUMP/μg of DNA. Therefore, in a series of human colon carcinomas, acid-soluble FdUMP formation, which ranged from 0 to 1.08 pmols/μg of DNA, was much more variable than was the case for Walker 256 carcinoma. One patient in this series, whose tumor formed only 0.10 pmol of FdUMP/μg of DNA, showed an antitumor response when treated with a combination of 5-FU plus methyl-CCNU subsequent to surgery.

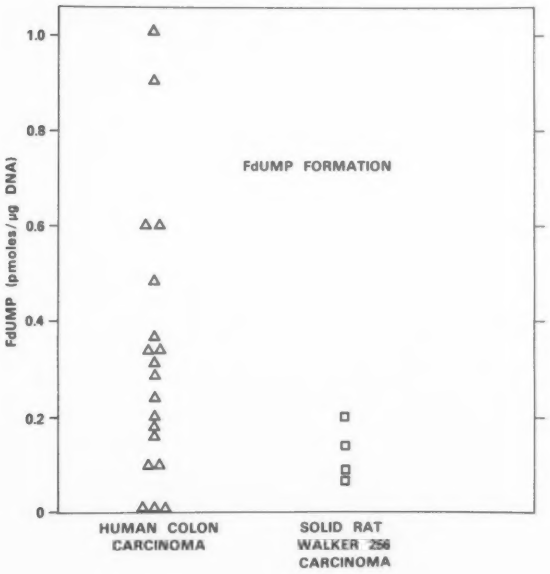

FIGURE 3.—Formation of FdUMP by Walker 256 and human colon carcinomas incubated with 200 μM 5-FU. Following incubation, tumor slices were washed, extracted, and analyzed for FdUMP with *L. casei* thymidylate synthetase as described in the Methods section. △ = human colon carcinoma samples; □ = rat Walker 256 carcinoma samples.

DISCUSSION

The object of this study was to determine if two hitherto unexamined biochemical effects of 5-FU in human colon carcinoma in vitro, namely, inhibition of ribosome synthesis and FdUMP formation, might be potential predictors of clinical antitumor response to 5-FU. We have shown in both human colon carcinoma and the transplantable solid rat Walker 256 carcinosarcoma that 5-FU inhibits ribosome synthesis, and that, in addition, the analog is anabolized to FdUMP. The inhibition by 5-FU of ribosome synthesis in Walker 256 and human colon carcinoma is consistent with the reports of 5-FU and/or 5-fluorouridine blocking the maturation of ribosomal RNA and hence ribosome synthesis in rodent cells (18,19). In addition, it is similar to our report of the effect of 5-FU on ribosome synthesis in *Bacillus cereus* (6).

In samples of human colon carcinomas treated in vitro with 5-FU, there was a wide variability in both the inhibition of DNA synthesis and FdUMP formation while there was little variability in the inhibition of ribosome synthesis. In contrast, in the rodent tumor, FdUMP formation as well as inhibition by 5-FU of DNA and ribosome synthesis were uniform and reproducible. This is not surprising since, in contrast to the human colon carcinomas we examined, the Walker 256 carcinomas consist of a much more uniform population of tumor cells.

The wide variability in the inhibition by 5-FU of ^{3}H-UdR incorporation into DNA in a series of samples of human colon carcinomas is similar in some respects to the results reported by Wolberg (8,9). This investigator observed a variability in a series of human colon carcinomas of the effect of 5-FU on incorporation of ^{14}C-formate into thymine DNA and ^{3}H-TdR into DNA. With respect to 5-FU inhibition of ribosome synthesis, in four human colon carcinoma samples we found that the magnitude of the effect was comparable with little variability. The apparently uniform effect of 5-FU which we observed may be related to the fact that the overall low rate of ribosome synthesis in samples of human colon carcinoma tended to decrease the sensitivity of the assay. This in turn would make it more difficult to discriminate between varying degrees of inhibition by 5-FU in a series of these

tumors. The fact that all four tumors showed a response to 5-FU is surprising since normally only a small percentage of tumors are susceptible to this drug.

Based on our results with samples of human colon carcinoma, the possibility of using sensitivity of ribosome synthesis to 5-FU as a clinical predictor does not appear promising. This is due in part to our observation of the relatively low rate of ribosome synthesis in the human tumors studied and the apparent lack of variability of effect of 5-FU. However, the system might be refined and the sensitivity increased if, for example, the inhibition of 5-FU on ribosomal RNA maturation (18) were compared in a series of human colon carcinoma samples.

Formation of FdUMP, considered to be the active metabolite responsible for the primary effect of 5-FU (3), might be a more likely candidate to test as a possible predictor of clinical response to 5-FU. In our series of 19 human colon carcinoma samples which were incubated with 5-FU, there was a wide variability in acid-soluble FdUMP formation. We were not able to determine whether there was a correlation between ^{3}H-UdR incorporation into DNA and FdUMP formation since each of these measurements was made in a separate series of colon carcinoma samples. Kessel and Hall (11), in a series of rodent tumors, showed that the increased responsiveness to 5-FU treatment paralleled the increased intratumor formation of 5-FU nucleotides. However, we could not determine whether those human colon carcinomas which formed larger amounts of FdUMP would be more responsive to 5-FU therapy.

Samples of human colon carcinoma suitable for our biochemical studies were obtained from 29 patients over an 18-month period. It would have been desirable to simultaneously measure in each sample the effects of 5-FU on ribosomal and DNA synthesis as well as FdUMP formation. However, only one biochemical effect of 5-FU could be measured in each tumor sample because of the limited amount of tissue we usually obtained. During our study, only two of the 29 patients from whom we obtained samples were subsequently treated with 5-FU alone, several patients received 5-FU together with other treatment, and, in fact, most patients received no chemotherapy at all. In addition, several patients were lost to the study so that followup was not possible. Thus, it was impossible to determine if there was a correlation between our in vitro results and the antitumor response to 5-FU.

The need for reliable in vitro predictors of clinical response of human colon carcinoma to 5-FU appears self-evident. Our study describes not only our rationale, but some of the practical difficulties in attempting to develop such a model. Based on our results, we would suggest that measurement of FdUMP formation in vitro might be undertaken at a cancer center(s) where large numbers of colon carcinoma patients undergo surgery and subsequent 5-FU therapy. It would also be of value to permit serial biopsies to evaluate tissue retention of this drug metabolite (17,20). Given such a situation, an answer with regard to the value of FdUMP formation as a predictor of clinical responsiveness to 5-FU might be ascertained in a reasonable period of time.

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Phase II Trial of Methotrexate in Carcinoma of the Bilharzial Bladder [1]

Brief Reports and Preliminary Communications

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Carcinoma of the bladder superimposed on chronic bilharziasis is predominantly of the squamous cell type (67%), with a minority showing transitional cell (23%), adenocarcinoma (8%), or unclassified (2%) histology (1). Few chemotherapeutic agents have been tested in this tumor. In clinical trials reported to date, only hexamethylmelamine has shown therapeutic promise (2). Because of activity in other forms of squamous cancer (3), a phase II trial of methotrexate (MTX) was undertaken in two regions where carcinoma of the bilharzial bladder is endemic. The results indicate that MTX has little useful clinical activity in this setting.

METHODS

This study was undertaken simultaneously at the Cairo Cancer Institute in Egypt and at the St. Francis' Hospital in Katete, Zambia. All patients had a histologic and/or cytologic diagnosis of bladder cancer superimposed on chronic bilharziasis. Patients were eligible for treatment according to the following criteria: (*a*) the tumor was inoperable stage T_3 (invasive, mobile) or T_4 (invasive, fixed) by the UICC classification (4); (*b*) the serum creatinine was < 1.5 mg/100 ml and the blood urea was < 40 mg/100 ml (Egyptian patients only); (*c*) both kidneys were functional by iv pyelogram; (*d*) patients had received no prior treatment of any kind; and (*e*) all patients were ambulatory (Karnofsky performance status > 70).

Treatment consisted of two similar regimens. In Egypt, MTX was administered im in doses of 50 mg daily × 3 days every 3 weeks for at least three courses. In Zambia, MTX was given orally in doses of 20 mg/m² daily × 4 days every 2 weeks for four courses. Dose reduction was scheduled in the Zambia trial as follows: 75% dose reduction for a urea level of 30–50 mg/100 ml and 50% dose reduction for a urea level of 50–100 mg/100 ml. Tumor measurements in three dimensions by recto-pelvic examination were recorded weekly in all patients throughout the trial. A partial response was recorded as a diminution of the product of two diameters by > 50%. Hemograms were repeated every 3–7 days and blood urea and urine cytology were repeated weekly. Patients were questioned for symptomatic change at weekly intervals.

RESULTS

Table 1 shows the results in 28 evaluable patients who completed at least three courses of MTX. In Egypt, 20 previously untreated patients had the following histologic distribution: squamous cell, 12; transitional cell, four; and undifferentiated cell, four. One patient (undifferentiated type) died 1 week after the first course of MTX; her blood urea rose to 180 mg/100 ml, and she developed jaundice, leukopenia, severe mucositis, and diarrhea. Of 14 patients treated in Zambia, all but one had squamous cell cancer. Five patients entered the trial but were either lost to followup (two patients), developed disease progression prior to the completion of three courses (two patients), or had intolerable toxic effects (one patient). Since the response trend was similar in both locales, the results are combined.

TABLE 1.—Results of 28 patients who completed at least 3 courses of MTX

Histology	No. of evaluable patients	No. of patients with— Partial response	Stable disease	Progression
Egypt				
Squamous	12	0	2	10
Transitional	4	0	0	4
Undifferentiated	3	1	0	2
Zambia				
Squamous	8	1	4	3
Transitional	1	0	1	0
Totals	28	2	7	19

[1] Received July 1, 1977; revised Mar 9, 1978; accepted Mar 22, 1978.

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Of 28 evaluable patients, only two partial, transient responses were observed, seven patients were felt to have stable disease, and 19 patients had progression while receiving therapy. At least half of the patients reported some symptomatic improvement during therapy.

Toxicity in this trial was considerable. All patients but one developed leukopenia and mucositis which were severe in > 50% of the patients. Diarrhea developed in over half the patients. At least one drug-related death occurred (*see* above).

DISCUSSION

While MTX shows considerable antitumor activity in squamous cell cancer of the head and neck, it does not appear to be clinically useful in squamous cell cancer of the bilharzial bladder in the doses and schedules employed in these trials. It is possible that high-dose regimens with citrovorum factor rescue may be more effective than conventional doses, but with bilharzial bladder cancer, this approach must be undertaken with considerable caution and careful monitoring.

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Response of Five Established Solid Transplantable Mouse Tumors and One Mouse Leukemia to Hyperbaric Hydrogen[1,2]

Brief Reports and Preliminary Communications

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Dole et al (1) reported that ultraviolet-induced squamous cell carcinomas underwent marked regression after a 10-day exposure of tumor-bearing mice to a mixture of 2.5% oxygen and 97.5% hydrogen at 8 atmospheres of pressure. We conducted similar studies using six transplanted tumors to determine if the tumor response reported by Dole et al could be obtained with other mouse tumors.

The tumors used in this study were the B16 melanoma, Mammary adenocarcinoma 16/C, Colon adenocarcinoma 38, Ridgway osteogenic sarcoma, Lewis lung carcinoma, and L1210 leukemia. These tumors cover a spectrum of histologic types and a variety of biologic behavioral characteristics.

At the stage of tumor advancement used in these studies, 29 of 34 antitumor agents reported to be clinically useful (2–4) showed cytocidal or cytostatic activity against one or more of these tumors.

MATERIALS AND METHODS

The histologic types and biologic characteristics of the tumors studied are listed in table 1 (5–7).

L1210 leukemia cells (10^3/mouse) were injected ip 1 day prior to the start of treatment. All other tumors were implanted sc by trocar as 20-mg fragments into the right axillary region. Just prior to starting treatment, animals with tumors from 200 to 700 mg in size were selected, randomized, and separated into two groups: one group was housed inside the hyperbaric hydrogen chamber and the other was held under ambient atmospheric conditions as untreated controls. Each mouse was individually identified, and the tumor weight and body weight were recorded on the first day of treatment. The mice were treated continuously for 7 days. After the mice were removed from the chamber, they were weighed once weekly and checked daily for death, and their tumors were measured twice weekly. The tumor weights were calculated from the two-dimensional measurements using the formula: tumor weight (mg) = $(a \times b^2)/2$, where a is the length (mm) and b is the width (mm). The method for hyperbaric hydrogen treatment has been reported previously (1).

The following quantitative endpoints were used to assess antitumor effectiveness: (*a*) percent increase in lifespan (% ILS) = 100 × (the median day of death of the treated mice, minus the median day of death of the control mice), divided by the median day of death of the control mice; (*b*) tumor growth delay, treated-control (T-C, days) = the time required for the treated tumors (median, 15) to reach 1250 mg, minus the time required for the control tumors (median, 15) to grow to the same size (this value was the single most important criterion of antitumor effectiveness and can be considered comparable to the duration of regression or the progression delay value used clinically); (*c*) tumor regression, partial (> 50%) and complete (below the limit of palpation).

RESULTS

The results of the first experiment are shown in table 2. The 15 animals in each group had no ILSs and no regressions in tumor size. The T-C values (–1.2 to 2.1) were in the inactive range.

In the second experiment (98.5% hydrogen and 1.5% oxygen at 13 atmospheres of pressure for 7 days), an excessive number of deaths occurred during decompression and no significant data were obtained except with mice bearing Mammary adenocarcinoma 16/C. This treatment effected a 3% ILS and a 2.1 T-C value, considered in the inactive range. No regressions in tumor size were observed.

A variety of transplantable mouse tumors were used in these experiments to determine the efficacy of hyperbaric hydrogen treatment. After 7 continuous days of hyperbaric hydrogen treatment, no inhibition of tumor growth was observed.

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TABLE 1.—Biologic characteristics of mouse tumors tested for response to exposure to hyperbaric hydrogen

Tumor	Origin	Histologic type	Germ layer origin of tumor	Yr of tumor origin	Mouse host			Exponential doubling time (days)	Metastasis*	Transplantation route
					Origin	Passage	Test			
B16 melanoma	Spontaneous	Melanoma	Ectoderm	1954	C57BL/6J	C57BL/6	BDF_1	1.1–1.7	+++	sc
Mammary 16/C	Spontaneous†	Adenocarcinoma	Ectoderm	1974	C3H/He	C3H	$B6C3F_1$	4.3–6.0	+++	sc
Colon 38	1,2-Dimethylhydrazine dihydrochloride	Adenocarcinoma	Entoderm	1973	C57BL/6	C57BL/6	$B6C3F_1$	5.0–6.0	+	sc
Ridgway osteogenic sarcoma	Spontaneous	Osteogenic sarcoma	Mesoderm	1948	AKm	AKR/Jax	$AKD2F_1$	2.6–3.4	–	sc
Lewis lung	Spontaneous	Carcinoma	Entoderm	1951	C57BL	C57BL/6	BDF_1	0.8–1.2	++++	sc
L1210	3-Methylcholanthrene	Leukemia	Mesoderm	1948	DBA/2	DBA/2	CDF_1	0.5		ip

*Metastasis rating: +++ and ++++ = highly metastatic; + = marginally metastatic; and – = nonmetastatic.
†Virus-associated.

TABLE 2.—Hyperbaric hydrogen treatment of mouse tumors

Tumor	Tumor size at start of treatment	Hyperbaric hydrogen treatment schedule*	Median day of death† (range of deaths in days)		% ILS	Duration of remission: T-C value	Activity rating‡
			Control	Treated			
B16 melanoma	500–1000 mg	Days 9–15	18.0 (14–30)	18.0 (16–30)	0	0.5	—
Mammary 16/C	200–500 mg	Days 13–19	52.0 (25–68)	55.0 (34–64)	5	0.3	—
Colon 38	200–500 mg	Days 19–25	67.0 (59–>80)§	67.0 (47–80)	0	–1.2	—
Ridgway osteogenic sarcoma	100–400 mg	Days 12–18	33.0 (19–46)	30.0 (21–52)	–10	–0.4	—
Lewis lung	200–500 mg	Days 7–13	32.0 (18–38)	29.0 (14–37)	–10	0.6	—
L1210	4×10^3 cells	Days 1–7	9.0 (9–10)	10.0 (9–10)	10		—

*Treatment: 97% H_2, 3% O_2, 8 atmospheres of pressure, 15 mice/group.

†Day postimplantation of tumor.

‡Activity rating of solid tumors for single course treatment:

Activity level	*T-C value (days)*
High	
++++	>19
+++	13–19
++	9–12
Marginal (+)	7–8
Inactive (–)	< 7

§7 control mice with far-advanced tumors were killed on Day 80.

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Glycerol: A Successful Alternative to Dexamethasone for Patients Receiving Brain Irradiation for Metastatic Disease[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Thirty-three patients with metastatic brain neoplasms of various types received glycerol instead of corticosteroids during periods of brain irradiation. In the 25 symptomatic patients, responses from this treatment were seen in those patients whose primary symptom was vomiting (ten of 12 patients), headache (nine of ten), papilledema (five of nine), paralysis (six of eight), confusion (six of seven), and dysphasia (four of six). Glycerol was well tolerated; it did not induce immunosuppression when administered in combination with radiotherapy and chemoimmunotherapy. Further investigation is indicated to compare its efficacy with that of dexamethasone.

[Cancer Treat Rep 62:1081–1083, 1978]

Metastases to the central nervous system are presently recognized more frequently in patients with hematologic malignancies and solid tumors, particularly in those patients with malignant melanoma and tumors of the lung and breast. These types of metastases are usually multiple and are treated with whole-brain irradiation and dexamethasone (1). However, the use of corticosteroids in the treatment of brain tumors has been detrimental to many patients, since steroids have an adverse effect on the body's immune system (2), and, therefore, the number of patients likely to benefit from some form of immunotherapy is reduced. This pilot study was conducted to evaluate the efficacy and toxicity of glycerol, an alcohol effective in the treatment of increased intracranial pressure due to a variety of causes (3–5), when administered to patients receiving radiotherapy to the brain for metastatic involvement.

MATERIALS AND METHODS

Thirty-three consecutive patients with biopsy-proven malignant disease and nonresectable brain metastases who were referred to the Department of Developmental Therapeutics at The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, were included in the study. After a detailed pretreatment medical, neurologic, and laboratory evaluation, the patients began to receive treatment with 50% glycerol orally. The daily dose of glycerol ranged from 1.2 to 2 g/kg given in four equal doses 6 hours apart. Each dose was mixed in 75 ml of unsweetened fruit juice to be consumed over a 15–20-minute period. Glycerol was given throughout the period of administration of brain irradiation and for the 2 weeks following it, and then was tapered off over a 3-day period. Twenty-four hours after starting glycerol, whole-brain irradiation was initiated. The total dose ranged between 3500 and 4500 rads and was administered usually in 20 dose fractions over a 10-day period. Twenty-four patients were able to receive additional systemic chemotherapy while receiving glycerol treatment. Seventeen patients received nonspecific immunotherapy and had periodic immunologic evaluations with a battery of skin tests utilizing six recall antigens (dermatophytin, Varidase, Monilia mix, mumps, keyhole-limpet hemocyanin, and purified protein derivative). The patients

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were followed closely with clinical, neurologic, and laboratory evaluations three times weekly. The results of the neurologic evaluation were recorded on a scoring chart, originally designed by Mathew et al (4). The patients were evaluated for neurologic improvement and changes in the immunologic status, in addition to tumor response.

RESULTS

Twenty-three of the 33 patients entered in the study had malignant melanoma. Most of the remaining patients had carcinoma of the lung and breast. Twenty-five percent of the patients were asymptomatic, and 75% of the remaining patients had moderate-to-severe neurologic manifestations at the time of presentation.

Nausea, vomiting, and headache were the most common presenting symptoms, and responses were seen in the patients whose primary symptom was vomiting (ten of 12) and headache (nine of ten). Papilledema, paralysis, and dysphasia were common neurologic signs, and a complete or partial response was obtained in patients with papilledema (five of nine patients), paralysis (six of eight), confusion (six of seven), and dysphasia (four of six). Responses were also seen in the small number of patients with cranial nerve palsy, seizure, and coma.

Analysis of the changes in the total scores of the patients measured at three time intervals, ie, pretreatment, 5–7 days, and 12–14 days after initiation of glycerol treatment, showed that the 15 patients with intracerebral metastases and minimal or no symptoms at presentation had no deterioration of their clinical status and maintained their near-normal mean scores. Fifteen patients with intracerebral metastases and moderate-to-severe symptoms had significant ($P < 0.006$) improvement during the first week, which continued during the second week ($P < 0.053$), when tested by the repeated-measures analysis (6). The three patients with meningeal carcinomatosis had a rapid improvement during the first week followed by stabilization of their condition.

The median survival of all patients treated with glycerol was 5.5 months. There was a significant difference ($P = 0.001$) in the survival of patients who had marked neurologic improvement (> 50% recovery of pretreatment neurologic deficit) or remained asymptomatic for 6 weeks from initiation of radiotherapy to the brain compared with the patients who failed to have significant neurologic improvement (< 50% recovery of neurologic deficit) or became symptomatic during the 6-week period following the initiation of radiotherapy.

The side effects of glycerol were related primarily to the gastrointestinal tract. Nausea and vomiting were seen in 35% of the patients on 15% of the days on which the drug was administered alone. These adverse effects were mild-to-moderate in most instances and were usually easy to control. Vomiting was severe in only four patients, necessitating temporary replacement of the drug with dexamethasone and resulting in rapid control of vomiting but no improvement in neurologic abnormalities. No significant metabolic or electrolyte abnormalities were seen in any of the patients.

DISCUSSION

Glycerol has been successfully used to control symptoms associated with increased intracranial pressure in patients with a variety of intracranial lesions (3–5). However, the experience reported with patients receiving brain irradiation is limited (5). The mechanism of action of glycerol is unknown, although it has been postulated that glycerol action is related to the increase in serum osmolality and the osmotic diuresis (3,7) associated with its administration. Recent studies show that glycerol increases the permeability of the blood-brain barrier (8,9) by producing widening of the tight junctions connecting the vascular epithelial cells. This property added to the capacity of glycerol to increase the cerebral blood flow and brain metabolism (6) could enhance the results of concomitant chemotherapy and brain irradiation.

The administration of glycerol was successful in preventing the development of cerebral edema due to radiation therapy in asymptomatic patients and in controlling and reversing the symptoms and signs of increased intracranial pressure in patients with metastatic brain disease. Unlike dexamethasone, glycerol treatment of up to 7 weeks was not associated with any significant metabolic or immunologic disturbances. Nausea or vomiting was the main toxic effect of glycerol, and it was not always possible to clearly establish the cause and effect relationship in view of the presence of other factors such as the simultaneous administration of irradiation or chemotherapy. As experience with using glycerol was gained, the incidence of nausea and vomiting decreased remarkably.

The lack of immunosuppressive properties during the period of glycerol administration was also of special significance. Specifically, six of the seven evaluable patients had either the same or a greater number of positive responses to six recall antigens in spite of concomitant administration of radiotherapy and chemotherapy, both of which have immunosuppressive effects. However, the concomitant

administration of immunotherapy may have influenced the results of skin testing in these patients.

We conclude that glycerol is effective in the treatment of edema secondary to radiation therapy to the brain. It can be administered safely in combination with radiotherapy and chemoimmunotherapy without compromising the immunity of the patient. Further investigation is required to compare the efficacy of glycerol with that of dexamethasone.

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Chemoimmunotherapy for Disseminated Malignant Melanoma: A Prospective Randomized Study[1]

Brief Reports and Preliminary Communications

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SUMMARY

Twenty-eight patients with disseminated malignant melanoma were treated with DTIC (250 mg/m² iv, Days 1–5) and actinomycin D (0.5 mg/day iv, Days 1–5) at 5-week intervals. Patients were randomly allocated to receive no immunotherapy or immunotherapy consisting of methanol extracted residue of bacillus Calmette-Guérin (0.5 mg intradermally; 100 μg in five separate sites) every 5 weeks, concomitant with chemotherapy. Of these 28 evaluable patients, 13 received chemoimmunotherapy with one complete response (CR) and 15 received chemotherapy alone with one CR. Both responses occurred in lymph node metastases. No partial responses were seen.

[Cancer Treat Rep 62:1085–1087, 1978]

DTIC is the most extensively studied and consistently active single agent in disseminated malignant melanoma. In several large studies, it has exhibited a rather narrow range of activity: 16%–31% (1–5). Actinomycin D has been examined as a single agent but results in disseminated malignant melanoma are conflicting. Molander and Oropeza (6) reported a 67% response rate in 24 patients, whereas Golomb et al (7) found no responses in 23 consecutive patients. Gerner et al (8) using 10-day infusions of both DTIC and actinomycin D every 4 weeks found a 20% objective response rate in 69 evaluable patients.

Gutterman et al (9) reported a nonrandomized study in which bacillus Calmette-Guérin (BCG) was combined with DTIC in disseminated disease. The results were compared to similar historical controls at their institution. A 27% response rate was noted in 89 patients compared to a 15% response rate in a historical control group of 111 patients receiving therapy with DTIC alone. The response rate of nodal metastases was greater (55%) for the combined therapy approach than for DTIC used alone (18%).

BCG preparations have proved difficult to standardize and the immune effects appear to vary with different strains, number of viable organisms, and ratio of viable to dead organisms. Because of this and the difficulties inherent in using living bacterial products and their systemic effects, attention has been directed toward nonviable microbial agents. Methanol extracted residue (MER)-BCG was developed in this line and described by Weiss (10).

MATERIAL AND METHODS

Patients

Twenty-eight patients with metastatic malignant melanoma were evaluated between February 1975 and December 1976. All patients had a histologically confirmed diagnosis of malignant melanoma and had metastatic disease either physically or radiographically measurable. No patients were receiving concomitant radiation therapy for their only measurable lesions. When treatment was started, all patients had not been receiving prior chemotherapy or radiation therapy for 2 weeks, wbc counts were $> 4000/mm^3$, platelet counts were $> 100{,}000/mm^3$, and BUN and serum creatinine values were < 25 and 1.5 mg/100 ml, respectively. The median age was 48 years for regimen 1 (chemotherapy alone) and 55 years for regimen 2 (chemoimmunotherapy). Regimen 1 included nine men and six women and regimen 2 included six men and seven women. All patients were white. Two patients (both in regimen 1) had received prior chemotherapy (methyl-CCNU). Eighteen patients had predominant

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visceral metastases (lung, bone, liver, brain, etc) and ten had predominant nonvisceral metastases (skin, subcutaneous, and lymph node). Regimen 1 included ten patients with predominant visceral metastases and five with predominant nonvisceral metastases. In regimen 2 there were eight patients with predominant visceral metastases and five with predominant nonvisceral metastases.

Patient Evaluation and Followup

Before each course of treatment, clinical examination, wbc and platelet counts, and hemoglobin levels were obtained for all patients. The measurement of the indicator lesion(s) was evaluated physically or radiographically at each visit.

Drug Administration and Dose Schedule

All patients received 5-day courses of DTIC at 5-week intervals (250 mg/m²/day iv) and actinomycin D (0.5 mg/day iv). We gave phenothiazines and/or barbiturates before treatment to inhibit nausea and vomiting or lessen its severity. New courses were given until progressive disease was detected. MER-BCG was given in doses of 100 μg intradermally in five separate sites every 5 weeks.

Assessment of Response

A complete response (CR) was defined as the disappearance of any tumor lesions and cancer symptoms. A partial response (PR) was defined as at least a 50% regression in the product of the longest perpendicular diameters of the indicator lesion(s). CR + PR was defined as objective response and had to be accompanied by significant subjective improvement. Responses had to last at least 4 weeks to be defined as such.

RESULTS

Therapeutic Response

The only objective responses seen were in two of ten patients with nonvisceral metastases. One patient receiving chemotherapy alone had a CR of bilateral hilar adenopathy. This response lasted 7 months from the time of the first treatment until progressive disease was documented. One patient had a CR with chemoimmunotherapy of a left axillary node. Complete resolution of adenopathy was not seen until 7 months after therapy and this followed a PR which had occurred at 4 months after therapy. Unfortunately, progressive disease developed only 2 months after CR had been obtained and 9 months after treatment started. None of the 18 patients with visceral metastases had objective response.

Toxicity

Gastrointestinal symptoms were the most prominent toxic effects, with nausea and vomiting experienced by all patients. Amelioration was obtained by pretreatment with phenothiazines and/or barbiturates, although nausea and vomiting in some patients was severe and led to discontinuation of therapy in two patients. The severity of nausea and vomiting lessened after each day of each treatment course. Hematologic toxic effects were mild, with no depressions of wbc counts to $< 3000/mm^3$ and platelet counts to $< 100{,}000/mm^3$ in only 6.6%. Toxic manifestations to MER-BCG were minor. Ulceration at injection sites was seen in 13% of the patients, but was always < 5 mm. Low-grade fever occurred in 13% of the patients but could also have been attributed to DTIC therapy.

DISCUSSION

The overall response to combined therapy with DTIC and actinomycin D in metastatic malignant melanoma in this study with or without immunotherapy was low. The treatment groups of chemotherapy versus chemoimmunotherapy were too small to evaluate the effect of MER-BCG, but the low objective response rates were not encouraging. We did find, as other recent studies have found, that toxicity from MER-BCG is acceptable. MER-BCG administration was given at the same time as chemotherapy in our study mainly for patient convenience because most of our patients drove $\geq$ 100 miles to be treated at our center. At the time of the design of our study in 1974, the most favorable conditions of dosage and timing of MER-BCG treatment had not been ascertained. In a recent article, Weiss (12) stated that these variables are still unknown. It is true from a theoretic standpoint that immunotherapy administration might be more beneficial at a time other than when chemotherapy is given. Our study was terminated because of poor responses to either protocol arm. Although each treatment arm had 13 and 15 evaluable patients, respectively, only one response was seen in each arm. The probability of obtaining objective response rates $> 30\%$ if a larger number of patients were subsequently entered is $\leq 5\%$ based on analysis from binomial distribution. Our studies seem to concur with those of Gerner et al (8) that actinomycin D in combination with DTIC has no benefit over the reported results with DTIC alone.

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Chemotherapy for Adenocarcinoma and Large Cell Anaplastic Carcinoma of the Lung With Ftorafur, Adriamycin, and *cis*-Dichlorodiammineplatinum(II)[1,2]

Brief Reports and Preliminary Communications

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The combination of *cis*-dichlorodiammineplatinum(II) (DDP) and adriamycin has been reported to give a 45% remission rate in 19 patients with large cell anaplastic carcinoma and adenocarcinoma of the lung (1). When 5-fluorouracil (5-FU) was combined with adriamycin, a 64% response rate was reported for 11 patients with the same tumor types (2). Ftorafur has a spectrum of antineoplastic activity similar to that of 5-FU, though it is significantly less myelosuppressive (3). It seems that ftorafur is slowly metabolized to 5-FU and produces a sustained high serum concentration of 5-FU (4,5), mimicking a continuous iv infusion of this compound. We were thus encouraged to choose ftorafur, adriamycin, and DDP in combination for the treatment of patients with adenocarcinoma and large cell anaplastic carcinoma of the lung.

PATIENTS AND METHODS

Consecutive patients with histologically proven inoperable adenocarcinoma and large cell anaplastic carcinoma of the lung who had not received prior therapy with any of the three chemotherapeutic agents and who had either measurable or evaluable disease were eligible for this study. Measurable lesions required the presence of clearly defined tumor margins so that the longest perpendicular diameters could be measured. Evaluable lesions were visible but not accurately measurable. Chemotherapy was given in 3-week cycles. Ftorafur at a dose of 3 g/day was infused over 2 hours on Days 1 and 4. Adriamycin at a dose of 60 mg/m^2 was given by rapid infusion on Day 1, and DDP at a dose of 15 mg/m^2/day was given in 500 ml of 5% dextrose solution over 4 hours on Days 1–4. Patients were encouraged to drink at least 3 quarts of fluid on the days of administration but no other means of hydration or forced diuresis were used to minimize nephrotoxicity. Patients had cbc, differential, and platelet counts measured at least twice weekly, and an SMA 12 blood chemistry profile and urinalysis were documented before each course of therapy to assess renal and liver function.

A complete remission (CR) required the disappearance of all clinical, radiologic, and laboratory evidence of tumor. A partial remission (PR) was defined as a $\geq 50\%$ decrease in the sum of the products of two perpendicular diameters of all measured lesions without simultaneous increase in the size of any lesion or the appearance of any new lesion. Stable disease was defined as a $< 50\%$ decrease in the size of measured lesions or progression $< 25\%$ for a period of at least 12 weeks without the appearance of new lesions. Progressive disease was defined as an increase of $\geq 25\%$ in the size of any measured tumor lesion or the development of any new lesions. Survival calculations were determined from the point of initiation of treatment by the method of Kaplan and Meier for censored and uncensored data (6). The differences between survival curves were evaluated by the one-tailed generalized Wilcoxon test according to Gehan (7).

RESULTS

Forty-seven patients entered the study. Two patients voluntarily withdrew before completing one course of therapy because of severe nausea and vomiting and are considered unevaluable. Two other patients died of tumor progression within 2 weeks of study entry but are included in the response and survival analysis. Five of the 45 evaluable patients had large cell anaplastic carcinoma and 40 had adenocarcinoma. The median age was 57 years (range, 29–73 years). All patients except four had either disease outside a single hemithorax or recurrent disease in a previously irradiated hemithorax. Twelve patients had received prior chest

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TABLE 1.—Response related to disease measurability; evaluable disease is visible but not accurately measurable

	No. of patients		
	Measurable disease	Evaluable disease	Overall
Total	24	21	45
CR	3 (42%)	—	3 (24%)
PR	7	1	8
Stable disease	8	13*	21 (47%)
Progressive disease	6	7	13 (29%)

* Includes 6 patients with tumor reduction < 50% but we were unable to measure further response.

radiotherapy. Twenty-one patients (69%) had a reduced performance status (≤ 70%, Karnofsky scale [8]) and 13 patients (30%) had lost ≥ 6% of their normal weight before therapy.

Response

Ten of 24 (42%) patients with measurable disease achieved either CR or PR. Eight measurable disease patients had stable disease as previously defined. Of the 19 patients with evaluable disease, one achieved at least 50% reduction in tumor size and six other patients achieved a definite shrinkage of tumor, but we were unable to accurately measure any further response (table 1). Failure to respond or achieve disease stability was significantly associated with a lower (≤ 70%, Karnofsky scale [8]) performance status ($P = 0.004$) and a pretherapy weight loss of ≥ 6% ($P = 0.02$).

Survival

The predicted overall median survival for the study was 24.6 weeks. Responders and patients with stable disease survived significantly longer than patients with progressive disease. The predicted median survival for patients with progressive disease was 15 weeks while the median survival for patients without progressive disease has not yet been reached. Increased survival was significantly related to a higher performance status and to a pretherapy weight loss of < 6% ($P = 0.01$ and 0.003, respectively). No significant difference in survival was found between patients achieving CR and PR and patients with stable disease ($P = 0.15$). Also, there was no difference between the survival of patients with measurable disease and those with nonmeasurable but evaluable disease ($P = 0.44$).

Toxicity

The median lowest recorded absolute neutrophil count was 900/mm^3 and the median lowest recorded platelet count was 154 × 10^3/mm^3 in 136 evaluable therapy courses. In general, myelosuppression was short in duration and occurred between Days 10 and 18. There was no evidence of cumulative myelosuppression in 21 patients who had five or more consecutive courses of therapy analyzed. Only 2% of patients required hospitalization for presumed or proven infection associated with neutropenia (< 1000 cells/mm^3). There were no drug-related deaths. In contrast, nausea and vomiting was the major toxic effect of this therapy. This was related both to the administration of ftorafur on Days 1 and 4 and to the added effect of DDP between the two doses of ftorafur. Fifty-five percent of all 135 analyzed courses of therapy were associated with persistent nausea and vomiting lasting for > 48 hours. In 6% of patients, vomiting was of such severity that parenteral rehydration was required. This regimen produced very few renal toxic effects and no ototoxic effects. Only three (6%) patients demonstrated a rise in serum creatinine > 1.4 mg/dl and had DDP withdrawn from at least one course of therapy. DDP was withdrawn from two or more consecutive courses in two patients and one of these had DDP permanently discontinued because of persisting renal function impairment. Although audiologic studies were not routinely done, there were no reports of hearing impairment or tinnitus.

DISCUSSION

This study further expanded our experience with the investigational chemotherapeutic agents ftorafur and DDP when used in combination with adriamycin. Apart from nausea and vomiting associated with therapy administration, the regimen was well tolerated. There was no problem with excessive myelosuppression and few nephrotoxic effects were observed. This is probably related to the bone marrow sparing effect of ftorafur (3) and the modest dose of DDP. A 42% response rate for patients with measurable disease compares favorably with other reported regimens. In a study using DDP combined with adriamycin and cyclophosphamide, Eagan and co-workers (9) reported that four of 12 (33%) patients with measurable adenocarcinoma and large cell anaplastic carcinoma of the lung responded. However, the addition of either ftorafur or cyclophosphamide does not appear to have significantly improved the response results of the DDP and adriamycin combination reported by Vogl et al (1) as mentioned previously.

Comparing the response rates reported for different studies on their face values may be misleading. In this study, patients with measurable disease had a 42% CR and PR rate whereas only one of 19 patients with evaluable disease could be considered a PR. However, like Eagan et al (9), we found no significant difference in survival between patients with measurable disease and patients with evaluable disease.

The need to investigate new therapies for lung cancer patients remains. The importance of pretherapy weight loss affecting both response to therapy and survival has led us to investigate nutritional replenishment with iv hyperalimentation for non-oat cell lung cancer patients. Active investigation with new chemotherapy and immunotherapy also continues.

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Phase II Study of Vinblastine, Methyl-CCNU, and Medroxyprogesterone in Advanced Renal Cell Cancer[1]

Brief Reports and Preliminary Communications

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SUMMARY

One hundred and sixty-five patients with advanced renal cell cancer were evaluable for combination or single-agent therapy with methyl-CCNU, vinblastine, and medroxyprogesterone. A low order of response was observed, and these agents were not proven effective as treatment for metastatic renal cell cancer. Performance status and a relatively long symptom-free interval from primary tumor to metastatic disease were found to be the most prognostically significant factors for survival.

[Cancer Treat Rep 62:1093–1095, 1978]

Patients with metastatic renal cell cancer represent a sizable proportion of patients with advanced urologic cancer. Patients with stage I and II surgically treated disease have a clearly improved 5-year survival although these patients represent a minority of the overall group of patients who have resectable disease. Despite aggressive surgical treatment, the overall 5-year survival rate remains at only 38%–40%. Progestational hormonal treatment trials for advanced disease have reported response rates ranging from 0 to 26% (1–3). Nonhormonal chemotherapy trials have been limited; these reports have cited small patient numbers with variable dose schedules and response criteria with a variety of agents (4–6). A review by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI, of published data suggests minimal or no activity for any of a number of agents which have been tested (7). This study is a prospective randomized program employing methyl-CCNU (meCCNU), vinblastine (Velban, Eli Lilly Co), me-CCNU-medroxyprogesterone (Depo-Provera, Upjohn Co), or vinblastine-medroxyprogesterone and was initiated by the ECOG as a phase II study to identify the antitumor activity of these regimens.

METHODS AND PROCEDURES

One hundred and ninety-seven patients with biopsy-confirmed adenocarcinoma of the kidney with measurable advanced or metastatic disease were randomized for treatment. Patients were stratified according to the grade of anaplasia of the tumor, age, performance status, primary site of metastatic disease, and previous treatment with a progestational agent. Patients having no previous progestational therapy were randomized to one of the four protocol treatment arms while patients with pre-

[1] Received Dec 9, 1977; revised Mar 21, 1978; accepted Mar 27, 1978.

[2] This study was performed by the Eastern Cooperative Oncology Group (ECOG) (*Paul P. Carbone*, chairman; CA-21115), and was supported by Public Health Service grants from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare. The following institutions participated in the study: Mayo Clinic, Rochester, Minn (*R. G. Hahn*; CA-13650); State University of New York, Buffalo (*N. R. Temkin* [*present address*: University of Washington, Seattle]; CA-12721); University of Rochester, NY (*E. D. Savlov*; CA-11083); Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill (*C. Perlia*; CA-10948); Medical College of Virginia, Richmond (*G. L. Wampler*; CA-10572); Albany Medical College, NY (*J. Horton*; CA-06954); and Yale University, New Haven, Conn (*J. Marsh*; CA-15485). Other participating institutions and principal investigators were: Paris Program (*L. Israel*); Ottawa Cancer Foundation (*D. Klaassen*); Jefferson Medical College (*H. Brodovsky*; CA-14215); Boston University Medical Center (*M. Straus*; CA-11109); Johns Hopkins University (*R. Lenhard, Jr*; CA-16116); Hahnemann Medical College (*J. Brodsky*; CA-13611); Tufts University School of Medicine (*L. Stolbach*; CA-07190); Jackson Memorial Hospital (*J. Colsky*; CA-13611); American Oncologic Hospital (*R. Creech*; CA-18281); University of Pittsburgh (*B. Fisher*; CA-18653); Lahey Clinic Foundation (*J. Bateman*); Northwestern University (*W. DeWys*; CA-17145); Albert Einstein College of Medicine (*B. Kaplan*; CA-14958); University of Pretoria (*G. Falkson*); University of Iowa; Brookdale Hospital Center (*M. Tobin*); University of Florida (*N. Abramson*; CA-19818); Chicago Medical School (*E. Ezdinli*; CA-14144); SUNY Stonybrook (*J. Archambeau*); Georgetown University (*B. Shnider*; CA-02824); Maimonides Medical Center (*J. Edmonson*); Pennsylvania Hospital (*H. Lerner*; CA-13613); Mt. Sinai Medical Center (*E. Greenspan*; CA-17152); NCI; Case Western Reserve University (*E. Monsour*; CA-14548); and University of Pennsylvania (*R. Cooper*; CA-15488).

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vious progestational therapy were randomized to meCCNU or vinblastine. MeCCNU was administered orally in a dose of 200 mg/m² every 7 weeks. Vinblastine was administered iv once weekly in a dose of 5 mg/m². Medroxyprogesterone was administered in a dose of 800 mg im once weekly in combination with the above meCCNU or vinblastine dose. Concurrent radiotherapy or other ongoing antitumor treatment precluded patient entry into the study. A re-evaluation to determine patient status and tumor response was scheduled 7 weeks after the start of therapy. Weekly wbc and platelet counts were scheduled after meCCNU therapy and before weekly vinblastine therapy. A 25% dose reduction for both of the cytotoxic drugs was provided if the wbc count nadir was < 2000/mm³ or if the platelet count nadir was between 25,000 and 50,000/mm³. Crossover randomization to one of the alternate single-agent or combination regimens was performed after failure with the initial treatment, if the patient was still a candidate for therapy. Objective tumor response was classified according to the usually employed criterion of 50% reduction in the sum of the perpendicular products of measurable disease. Bone scans, liver scans, and other ancillary laboratory tests were not considered measurable criteria for disease response.

PATIENT CHARACTERISTICS

One hundred and ninety-seven patients were entered in this study from December 1972 to January 1975; only 165 of the 197 randomized patients had adequate followup data for analysis of tumor response. Thirty-two patients were excluded from the main analysis: ten patients did not receive the scheduled treatment, six had no followup information, six had an initial performance status of 4 (totally bedridden), three were not randomized, three had no measurable disease, one died before starting treatment, one had an initial diagnosis which was changed to a different primary cancer, one received the wrong treatment, and one was cancelled from treatment. Patients who died within the first month or who had treatment discontinued because of toxicity or clinical progression were considered evaluable for response. Ninety (54.5%) patients had metastatic pulmonary disease listed as the measurable metastatic disease, 18 (11%) had osseous disease, 11 (6%) had liver disease, and 38 (23%) had other measurable disease. The metastatic disease site was not specified for eight patients. Of the 165 treatment-evaluable patients, primary treatment randomizations were as follows: the meCCNU-medroxyprogesterone arm, 38 patients; the vinblastine-medroxyprogesterone arm, 38 patients; the meCCNU-only arm, 45 patients; and the vinblastine-only arm, 44 patients. Sixty-seven patients were crossed over to alternate treatment after failure with the initial treatment arm. Five patients of this group were assigned to the meCCNU-medroxyprogesterone arm, eight to the vinblastine-medroxyprogesterone arm, 33 to the meCCNU-only arm, and 21 to the vinblastine-only arm. Sixty-two patients receiving the crossover phase had adequate followup data for determination of tumor response.

TOXICITY

Hematologic toxicity was more severe with the two meCCNU regimens. One instance of lethal pancytopenia occurred with the meCCNU-medroxyprogesterone arm. Eighteen additional instances of life-threatening hematologic toxic effects (wbc count < 1000/mm³ or platelet count < 30,000/mm³) occurred. Nine of these patients were receiving meCCNU-medroxyprogesterone, eight were receiving meCCNU alone, and one was receiving vinblastine-medroxyprogesterone. Nine patients had bleeding associated with the thrombocytopenia, three with the meCCNU-only arm and six with the meCCNU-medroxyprogesterone arm. All regimens but the vinblastine-medroxyprogesterone arm produced substantial vomiting. Neurotoxicity was confined to the vinblastine regimens.

RESPONSE RATES AND SURVIVAL BY TREATMENT AND AUXILIARY VARIABLES

The objective tumor response rate of each of the different treatment arms included in this study is given in table 1. The response rate (< 11%) is low with each of the treatments. None of the treatment comparisons is statistically different. No consistent relationship among response rates identifies an advantage to any of the combinations or single agents employed. None of the auxiliary factors considered (grade of tumor, age, site of metastases, prior progestational therapy) were particularly helpful in predicting response. Patients who were capable of normal activity (performance status of 0) had a significantly higher response rate and a longer survival period than nonambulatory or poor performance status patients. Patients with longer intervals between first symptoms and study entry had better survival than those with shorter intervals. Failure was rapid with all of these regimens in

TABLE 1.—Objective tumor response to therapy

Response	No. of responses (%) with—				
	MeCCNU + medroxy-progesterone	Vinblastine + medroxy-progesterone	MeCCNU	Vinblastine	Totals
Complete	1 (3)	0 (0)	0 (0)	2 (5)	3 (2)
Partial	3 (8)	3 (8)	2 (4)	2 (5)	10 (6)
No change	4 (11)	5 (13)	8 (17)	3 (7)	20 (12)
Progression	26 (68)	25 (66)	30 (67)	34 (77)	115 (70)
None	4 (11)	5 (13)	5 (11)	3 (7)	17 (10)
Totals	38	38	45	44	165

that > 50% of the patients had disease progression within 3 months after entering the study.

DISCUSSION

Preliminary reports suggesting antitumor activity of CCNU and vinblastine in patients with adenocarcinoma of the kidney prompted the choice of me-CCNU, a potentially more active nitrosourea, and vinblastine as the cytotoxic agents. Unfortunately, neither the single-agent treatment nor the addition of progestational therapy employing medroxyprogesterone provided any meaningful antitumor treatment to the overall group of patients involved in this study.

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Failure of Moderate-Dose Prolonged-Infusion Methotrexate and Citrovorum Factor Rescue in Patients With Previously Treated Metastatic Neuroblastoma—A Phase II Study [1]

Brief Reports and Preliminary Communications

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This study, which is one of a series of phase II studies conducted by the Children's Cancer Study Group in children with previously treated metastatic neuroblastoma, is a further attempt to identify single agents or drug combinations which demonstrate effectiveness in this generally resistant tumor. The objective of these studies is to find several active agents which might be combined in a phase III study for newly diagnosed neuroblastoma.

Since successes have been reported recently with high-dose methotrexate (MTX) with citrovorum factor (CF) rescue in patients with osteogenic sarcoma (1), head and neck cancer (2), lung tumors (3), and leukemia (4), it was decided to investigate a similar treatment program in patients with previously treated refractory neuroblastoma.

By measuring tritiated thymidine incorporation into metastatic neuroblastoma in vivo, Wagner and Käser (5) determined that approximately 11% of the cells were in S phase at any one time and that the mean duration of S phase was 27–28 hours. Therefore, we postulated that if neuroblastoma cells were exposed to cytocidal levels of an S-phase active agent for ≥ 28 hours, a maximal cell-kill for those cells in S phase during the infusion might be achieved.

The cytocidal MTX concentration for neuroblastoma is not known, but levels of about 1×10^{-6} M have been shown in vivo to inhibit DNA synthesis in leukemic cells (6). Bleyer[3] has demonstrated in a pilot study that 5×10^{-6} M MTX can be tolerated for as long as 42 hours before CF rescue without serious toxicity.

Based on these considerations, this study was designed to maintain a plasma MTX level of 5×10^{-6} M for 30 hours, a safe and possibly effective period, after which CF would be started and continued until the plasma MTX concentration was $< 5 \times 10^{-8}$ M.

MATERIALS AND METHODS

Eligibility

Patients entered in this study must have had previously treated and progressive metastatic neuroblastoma. Infants with metastasis limited to liver, bone marrow, or skin with small stage I or II primary tumors were excluded. Patients were expected to live ≥ 4 weeks and to complete at least two full courses of therapy. Only patients with at least one measurable lesion that had not been treated by X-ray therapy were entered in the study.

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[2] This study was conducted by the Children's Cancer Study Group (CCSG). The following institutions (with investigators and grant support) participated in the study: University of Southern California, Los Angeles (*G. D. Hammond, J. Weiner, R. Honour,* and *H. Sather,* CA-13539); University of Michigan, Ann Arbor (*R. Heyn,* CA-02971); Children's Hospital of the District of Columbia (*S. Leikin,* CA-03888); Cornell Medical Center, New York, NY (*D. R. Miller,* CA-14557); Children's Hospital of Los Angeles, Calif (*G. Higgins,* CA-02649); Babies Hospital, New York, NY (*J. A. Wolff,* CA-03526); Children's Hospital of Pittsburgh, Pa (*V. Albo,* CA-07439); Children's Hospital of Columbus, Ohio (*W. A. Newton,* CA-03750); Children's Orthopedic Hospital, Seattle, Wash (*R. L. Chard, W. A. Bleyer,* and *J. R. Hartmann,* CA-10382); University of Wisconsin, Madison (*N. Shahidi,* CA-05436); University of Minnesota, Minneapolis (*M. E. Nesbit,* CA-07306); Children's Memorial Hospital, Chicago, Ill (*G. Honig,* CA-07431); University of Utah Medical Center, Salt Lake City (*M. E. Lahey,* CA-10198); Princess Margaret Hospital, Toronto, Canada (*M. J. Sonley,* Ontario Cancer Treatment and Research Foundation grant 41–17); University of Rochester, NY (*M. Klemperer,* CA-11174); Children's Hospital of Louisville, Ky (*D. R. Kmetz*); University of British Columbia, Vancouver, Canada (*J. M. Teasdale,* Vancouver Foundation); Children's Hospital of Philadelphia, Pa (*A. E. Evans,* CA-11796); Indiana University, Indianapolis (*R. Baehner,* CA-13809); New Jersey College of Medicine, Newark (*L. Vitale,* CA-12637); Harbor General Hospital, Torrance, Calif (*J. Z. Finklestein,* CA-14560); University of California, San Francisco (*A. Ablin,* CA-17829); and Rainbow Babies and Children's Hospital, Cleveland, Ohio (*S. Gross*). All CA grants are from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

**Reprint requests to:* CCSG Operations Office, 1721 N Griffin Ave, Los Angeles, Calif 90031.

[3] Bleyer WA. Minutes of the New Drug Liaison Meeting (Bono VT, ed). National Cancer Institute, Bethesda, Md, Feb 1977.

Informed consent to enter the study was obtained from the patient's parents or guardian. Prior to treatment, patients were required to have a total neutrophil count $\geq 1500/mm^3$ and a platelet count $\geq 75{,}000/mm^3$. Any neutrophil or platelet count was acceptable when tumor infiltration of the bone marrow was present.

Treatment Program

At the beginning of the study, the first dose of MTX was 1000 mg/m^2 except for two patients who were started at 500 mg/m^2. It became apparent that there was minimal toxicity at this dose, so the starting dose was raised to 2000 mg/m^2 of MTX. In the first hour, 15% of the total dose was infused and the remaining dose was given over the next 29 hours. Courses were repeated every 2 weeks. As long as toxicities were acceptable each course was increased by a total of 500 mg/m^2 to a maximum total dose of 3000 mg/m^2. At the completion of the MTX infusion (30 hours from the start of treatment), im or iv CF (9 mg/m^2) was administered every 6 hours for a total of ten doses or until the plasma levels of MTX were $< 5 \times 10^{-8}$ M. Provision was made for alteration of the dose and duration of CF therapy when the serum creatinine level was elevated or when severe mucositis, myelosuppression, or hepatic toxicity occurred.

RESULTS

Twenty patients were entered in the study from May 1976 to February 1977. Four patients were considered unevaluable, since they had died or were taken off the study for progressive disease after only one course of treatment.

Of the 16 evaluable patients, none had complete or partial remissions. Four patients died shortly after two courses of therapy and one patient died after three courses. Eleven patients were taken off the study because of progressive disease and one was taken off because of no response. One patient who received five courses of therapy and another who received seven courses had subjective relief of pain with apparent improvement but without objective response. The median number of courses of MTX for evaluable patients was two (range, two to seven courses) (table 1). The median maximum dose was 1500 mg/m^2 of MTX (range, 1000–3000 mg/m^2) (table 2).

Thirteen of the 16 evaluable patients had no significant hematologic toxicity. Three patients, all with bone marrow disease prior to treatment, had marked myelosuppression after the MTX. In one patient receiving a dose of 500 mg/m^2, the platelet count fell from 200,000 to $12{,}000/mm^3$ in 17 days. In another patient receiving a dose of 1500 mg/m^2, the wbc count fell from 6200 to $950/mm^3$ and the platelet count fell from 42,000 to $7000/mm^3$ in 5 and 7 days respectively. In the third patient, receiving a dose of 1000 mg/m^2, a fall in the platelet count from 185,000 to $41{,}000/mm^3$ occurred 8 days after the drug dose. This patient had a large pleural effusion which contained measurable MTX 6 days after its administration. The treatment was judged to be at least partly responsible for the myelosuppression.

Ten of the 16 patients had no gastrointestinal toxicity while in five the toxicity was mild and in one the toxicity was severe.

Plasma MTX was assayed in 15 courses. In 11 courses the MTX levels were $\geq 5 \times 10^{-6}$ M at 30 hours. The lowest level, 2×10^{-6} M at 30 hours, was reported twice at doses of 500 mg/m^2 and once each at doses of 2000 and 3000 mg/m^2. All patients reported had levels $< 5 \times 10^{-8}$ M after ten doses of CF.

TABLE 1.—No. of courses of MTX per patient

No. of courses	No. of patients
1*	4
2	9
3	4
4	1
5	1
6	0
7	1

*Not considered evaluable.

TABLE 2.—Maximum dose of MTX in 16 evaluable patients

MTX dose (mg/m^2)	No. of patients
1000	3
1500	8
2000	2
2500	1
3000	2
Total	16

DISCUSSION

All 16 patients treated with moderate-dose MTX (median dose, 1500 mg/m^2) in a 30-hour infusion followed by CF rescue for a median of two courses separated by 2 weeks failed to have any objective response to therapy. When given at this dose and by this method, MTX-CF is ineffective for previously treated metastatic neuroblastoma.

It can be reasonably asked if higher doses would have improved the response. A higher dose would be possible if greater toxicity were acceptable. At doses of MTX $\leq$ 1500 mg/m^2 there were three cases of moderate hematologic toxicity attributable at least in part to the therapy, although all occurred in patients with bone or bone marrow infiltration. One patient was judged to have severe gastrointestinal toxicity manifest by bleeding oral ulcers after an MTX dose of 1000 mg/m^2. Five other patients had mild toxicity manifest by oral ulcers. In all 11 courses of MTX $\geq$ 2000 mg/m^2 there was no reported hematologic or gastrointestinal toxicity. However, since only two patients had a subjective response and none had an objective beneficial response, it was our judgment that further escalation of dose or duration of infusion would result in greater toxicity without significant enhancement of antitumor activity. A majority of patients had plasma MTX concentrations $> 5 \times 10^{-6}$ M, the plasma level which we postulated might be effective. This, however, was based on in vivo inhibition of DNA synthesis in leukemic cells and may not be relevant to neuroblastoma cells in vivo.

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Piperazinedione in Patients With Advanced Malignant Melanoma: A Southwest Oncology Group Study [1,2]

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SUMMARY

Piperazinedione was administered in doses of 9 or 12 mg/m² by iv infusion every 3 weeks in 28 patients with previously treated malignant melanoma. Of the 25 evaluable patients, 72% had drug-induced toxicity: 40% had leukopenia, 56% had thrombocytopenia, 40% had anemia, and 16% had nausea and vomiting. None of these patients had partial remission, two had stable disease, and the remaining 23 had definite progression of their disease in spite of adequate trial with this agent.

[Cancer Treat Rep 62:1101–1103, 1978]

Piperazinedione is one of the recent piperazine derivatives which were introduced into clinical trial as antitumor agents in the 1960s. It has shown activity against L1210 leukemia, P388 leukemia, B16 melanoma, Walker 256 carcinosarcoma, Lewis lung carcinoma, and ependymoblastoma (1). It may inhibit DNA synthesis and may act as an alkylating agent.

In a pilot study of piperazinedione in doses ranging from 1.5 to 36.0 mg/m² iv every 3–4 weeks, Gottlieb et al (2) reported clinical responses in patients with previously treated malignant melanoma. Of 16 evaluable patients treated, there were two partial remissions (PR) and one stable disease. The duration of the two PRs was 2+ months and 1 month. These investigators recommended a dose of 12 mg/m² for clinical trials. The SWOG started a phase II trial of piperazinedione in previously treated patients with disseminated malignant melanoma on October 28, 1976.

MATERIALS AND METHODS

Patients with histologically confirmed advanced malignant melanoma with a measurable lesion(s), who failed with first-priority chemotherapy trials, were included in this study. Patients should have had a minimum expected survival of 8 weeks, should have had no chemotherapy for at least 3 weeks prior to entry in this study, and should have recovered from prior toxic reactions. The following studies were performed before the start of therapy: cbc and platelet counts, bone marrow aspiration and needle biopsy, urinalysis, creatinine, BUN, glucose, uric acid, alkaline phosphatase, SGOT, calcium, bilirubin, prothrombin time, and chest roentgenogram. Other studies were performed as indicated.

The initial dose of piperazinedione was 12 mg/m² for patients with adequate bone marrow reserve or 9 mg/m² for those with inadequate bone marrow reserve (> 65 years old and/or previous radiotherapy or extensive chemotherapy). The drug was given as a rapid iv infusion over 15–30 minutes and was repeated every 3 weeks, or until the nadirs of blood cell counts had been reached and the counts were recovering (granulocytes $\geq 2000/mm^3$ and platelets $\geq 100{,}000/mm^3$). Doses of piperazinedione were subsequently adjusted in relation to hematologic toxic effects (table 1).

During therapy, cbc and platelet counts, other laboratory tests, and roentgenograms were performed to evaluate drug toxicity and disease status.

RESULTS

At the close of the study on September 20, 1977, 28 patients had been included, 21 male and seven female patients with an age range of 19–71 years (median, 56 years). All patients except one had had

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[3] This study was conducted by the Southwest Oncology Group (SWOG). The following institutions participated in the study: Wayne State University School of Medicine, Detroit, Mich (*M. Al-Sarraf*); University of Mississippi Medical Center, Jackson (*T. Thigpen*); Cleveland Clinic Foundation, Ohio (*C. W. Groppe*); University of Arkansas Medical Center, Little Rock (*A. Haut*); and Veterans' Administration Hospital, Little Rock, Ark (*F. Padilla*).

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TABLE 1.—Dose adjustment made according to hematologic toxic effects

Degree	Wbc count (cells × 10^3/mm^3)	Platelet count (cells × 10^3/mm^3)	Adjustment
None	≥ 4.0	≥ 100	12 to 15 mg/m^2
Mild	3.0–3.9	75–99.9	Same dose
Moderate	2.0–2.9	50–74.9	9 to 6 mg/m^2
Severe	1.0–1.9	25–49.9	6 to 4.5 mg/m^2
Life-threatening	< 1.0	< 25	Therapy discontinued

TABLE 2.—Incidence and severity of piperazinedione side effects in 25 patients

	No. of patients with—			
Degree	Leukopenia	Thrombo-cytopenia	Anemia	Nausea and vomiting
Mold	4	3	5	2
Moderate	4	6	5	1
Severe	2	4	0	1
Life-threatening	0	1	0	0
Total	10 (40%)	14 (56%)	10 (40%)	4 (16%)

previous surgery: 27 had removal of the primary lesion, 11 had regional lymph node dissection, and seven had removal of distant metastatic sites. Four patients had had previous radiotherapy for symptomatic metastatic disease and all had had first-line chemotherapy with DTIC alone or in combination with other cytotoxic agents. Ten patients had received a second line of chemotherapeutic drugs.

Twenty-five patients were considered fully evaluable for response and drug toxicity. Three patients were partially evaluable, two because of early death and one because he refused therapy after one dose of piperazinedione and was lost to followup.

Fifteen patients had an adequate bone marrow reserve and received an initial drug dose of 12 mg/m^2 iv. In the remaining 13 patients, the initial dose of the drug was 9 mg/m^2 iv. Ten patients received one dose of piperazinedione therapy, 14 had two doses, three had three doses, and only one had four doses. Five of the 28 patients are still in the study. The total dose given ranged from 15 to 65 mg with a median of 33.5 mg.

Drug Toxicity

The major toxic effect of piperazinedione was bone marrow suppression as manifested by leukopenia, thrombocytopenia, and anemia (table 2). Seven (28%) of 25 patients experienced no toxic effects while 18 (72%) had one or more toxic effects from piperazinedione. One patient had life-threatening thrombocytopenia, four had severe thrombocytopenia, and two had severe leukopenia. No drug-related deaths occurred. Nausea and vomiting were manageable.

The range of the lowest wbc count was 12–40 days (median, 22 days) after one dose and 19–38 days (median, 21.5 days) after two doses. Recovery from leukopenia ranged between 21 and 52 days (median, 27.5 days) after the first dose and between 28 and 41 days (median, 31 days) after the second dose. The time to the platelet count nadir was 15–50 days (median, 20 days) after one dose and 19–33 days (median, 21 days) after two doses, with recovery between 22 and 55 days (median, 39 days) after one dose and between 20 and 36 days (median, 33 days) after two doses.

Response

None of the patients had objective tumor response from piperazinedione in this study; two had stable disease and the rest had definite progression of their measurable disease or the appearance of new lesions in spite of adequate therapy with this agent.

DISCUSSION

Phase I–II studies of piperazinedione reported by Gottlieb et al (2) in doses ranging from 1.5 to 36 mg/

m^2 every 3 weeks produced clinical response in malignant melanoma (two PRs and one stable disease among 16 evaluable patients), acute myelocytic leukemia (six of six improved), chronic myelocytic leukemia (two of two had PR), and improvement of disease status in prostate (one of one) and renal cell carcinoma (one of one). Stable disease was reported in colon (two of five patients), head and neck (squamous cell) (one of five), adenocarcinoma of unknown primary (one of three), pancreas (one of one), and thyroid carcinoma (one of one).

Piperazinedione in combination with cyclophosphamide and DTIC produced severe myelosuppression in ten patients with solid tumors who had been previously treated, three of whom had malignant melanoma (3).

The drug-induced toxicity observed in this phase II trial was dose-related and the degree and incidence of the hematologic side effects were comparable to those previously reported. In our patients, the median day of the lowest wbc count in leukopenic patients was 22 days and the median day of the lowest platelet count in thrombocytopenic patients was 20 days. These median values are 4–5 days longer than those reported by Gottlieb et al (2). No drug-related deaths occurred and none of our patients had objective tumor regression.

It is concluded that piperazinedione therapy is inactive in previously treated patients with disseminated malignant melanoma.

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LETTERS

Instability of *cis*-Dichlorodiammineplatinum in Dextrose Solution[1]

cis-Dichlorodiammineplatinum (CDDP) is a neutral coordination complex of platinum which has significant antineoplastic activity in a variety of human and animal tumors. Nationwide phase II protocols are presently evaluating the efficacy of CDDP in specific tumor types and in combination with other agents (1). Most published studies have used injections of CDDP supplied by the National Cancer Institute (NCI), Bethesda, Md.[2] When reconstituted with sterile water for injection, this preparation contains CDDP in normal saline solution with 1% mannitol, suitable for direct iv bolus administration.

Recent work has focused attention on the benefits of concurrent hydration and forced diuresis in amelioration of renal toxicity due to CDDP. These studies may prompt clinical investigators to administer infusions of CDDP, diluted in large volumes of isotonic solution. The chemical behavior of CDDP (2) may create a significant drug instability problem under these conditions. An experiment was undertaken to assess the practical importance of CDDP instability in dilute aqueous solutions, and to bring this phenomenon to the attention of investigators who are involved in the design of clinical protocols.

CDDP, manufactured by the Ben Venue Laboratories, Inc, Bedford, Ohio (lot BV-76-263), was obtained from the NCI. Each vial contained CDDP (10 mg), mannitol (100 mg), and sodium chloride (90 mg). To produce the CDDP stock solution, vials were stored at 4°C and were reconstituted with 10 ml of nonsterile glass-distilled water just prior to use.

Five ml of CDDP stock solution was diluted with sterile 5% dextrose injection, USP, or with sterile 0.9% sodium chloride injection, USP (Travenol Laboratories), to a volume of 50 ml. Solutions were then aged at ambient temperature in the dark. Ten-ml aliquots were removed and frozen at intervals. The aliquots were later lyophilized to dryness and then reconstituted with 2 ml of glass-distilled water. A 20-μl sample from each aliquot was spotted on Whatman No. 1 filter paper and air-dried. A 10-μl sample of freshly prepared CDDP stock solution was spotted in the control lane. The chromatograms were developed using a system modified after Basolo et al (3). Chromatograms were air-dried and spots were visualized by spraying with 20% stannous chloride in concentrated hydrochloric acid.

TABLE 1.—Aquation of CDDP in chloride-free acid solution*

Equilibrium constants and half-lives of reactions

Reaction (fig 1)	K_{EQ}	Half-life (hrs)
I → II	3.3×10^{-3}	7.7
II → III	4.0×10^{-4}	5.8

Equilibrium concentrations of aquation species

Species (fig 1)	Concentration (M)	
	Initial	Equilibrium
I	2.5×10^{-3}	0.83×10^{-3}
II	0	1.36×10^{-3}
III	0	0.31×10^{-3}

*Data from ref 4.

This experiment demonstrated that CDDP is stable in physiologic saline for ≥ 24 hours (R_f = 0.56). In contrast, CDDP diluted in 5% dextrose is converted to two breakdown products with R_f values of 0.39 and 0.32 after < 2 hours of aging in the dark at room temperature. The chemical identification and pharmacologic evaluation of these materials have not been done.

CDDP in aqueous solution is initially a neutral

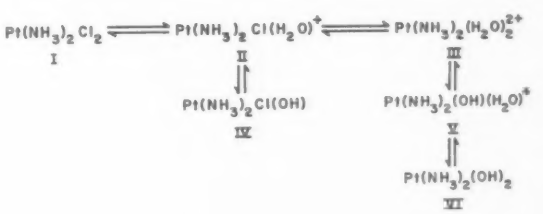

FIGURE 1.—Aquation reactions of CDDP(I) in chloride-free aqueous solution.

[1] Supported in part by institutional research grant IN-40-Q from the American Cancer Society. Dr. Earhart is a recipient of Junior Faculty Clinical Fellowship 388 from the American Cancer Society.

[2] Handelsman H, Goldsmith MA, Broder LE, et al. Clinical brochure: *cis*-platinum (II) diamminedichloride (NSC-119875). Prepared by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI, Bethesda, Md, 1974.

species, but a gradual increase in conductivity occurs over time (4). The chloride ligands are sequentially replaced by aquo ligands to produce this effect. Under physiologic conditions, hydrogen ions can be lost to convert the aquo ligands to stable, neutral hydroxyl ligands, according to the reaction scheme shown in figure 1. It has been proposed that the diaquodiammine platinum species (III) is the moiety responsible for the antineoplastic activity of CDDP (2). High extracellular chloride levels favor the extracellular persistence of CDDP(I), which as a neutral species can readily cross cell membranes. Low intracellular chloride favors the aquated species (III), generating the active form intracellularly. Extracellular charged species (II, III, and V) probably do not reach intracellular sites of action readily, and hydroxyl-containing species (IV and VI) are probably inactive as antineoplastic agents (2). If CDDP is converted to these species prior to crossing the cell membrane, its antineoplastic effectiveness may be reduced.

The kinetic constants measured by Reishus and Martin (4) (table 1) have been used to compute the expected acid equilibrium concentrations of each of the species. The predominant species in chloride-free acid solutions is the monochloro, monohydroxy form (II). Under these conditions, the reactions are relatively slow, with half-lives of 7.7 and 5.8 hours for the respective aquation steps.

The pK_A for the first hydrogen ion of the diaquo species (III) is 5.46 and the pK_A for the second hydrogen ion is 7.16 (4). The pK_A of II is estimated at 7.4. Thus, an equilibrium solution of CDDP in water at pH 6 will contain a mixture of compounds, with I, II, and V as major components. If CDDP stock solution is added to aqueous 5% dextrose at pH 6, the small amount of chloride present in the CDDP stock solution will alter these equilibria somewhat, but a significant conversion of CDDP to other species must occur.

In the clinical setting, hydration of a patient often involves administration of large volumes of isotonic iv fluids concurrently with CDDP. Sodium restriction may be desirable in individual patients with concomitant cardiac disease. Optimal use of CDDP may entail slow infusions rather than bolus doses. These factors may suggest that CDDP should be diluted in large volumes of solution prior to administration. The present study indicates that CDDP is not stable when diluted in low-chloride aqueous solution, and probably should not be diluted in solutions containing < 160 mEq/liter of chloride ion. Interpretation of results from studies which violate this rule must be done with care, since the clinical effectiveness of CDDP may be reduced under these conditions.

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A Single Dose of Metoclopramide in the Control of Vomiting From *cis*-Dichlorodiammineplatinum(II) in Man[1]

Metoclopramide has been used in some diagnostic studies of the gastrointestinal tract, in the treatment of vomiting of various etiology, and in a variety of functional and organic gastrointestinal disorders. It has been suggested that metoclopramide produces its antiemetic effect by blocking the chemoreceptor trigger zone for vomiting. An uncontrolled study by Boisson and Albot (1) suggested its effectiveness in controlling emesis induced by a variety of drugs in > 80% of their patients. However, Moertel and Reitemeier (2) in a double-blind controlled study utilizing placebo and phenothiazine in patients with advanced carcinoma of the stomach and pancreas, who were receiving a combination of chemotherapeutic agents that consisted of 5-fluorouracil, mitomycin C, and BCNU, reported that no antiemetic activity could be attributed to metoclopramide.

Repeated administration of metoclopramide could result in mild and reversible side effects on discontinuation of the drug. Such side effects include lassitude, drowsiness, dizziness, faintness, bowel disturbances, and, very rarely, extrapyramidal effects.

cis-Dichlorodiammineplatinum(II) (DDP) is a chemotherapeutic agent that, when administered iv in man, can induce severe nausea and vomiting

[1] Metoclopramide: 4-amino-5-chloro-*N*-[2-(diethylamino)ethyl]-o-anisamide monohydrochloride. Supplied by A. H. Robins Co, Richmond, Va (AHR-3070).

which does not respond to various antiemetic agents. This letter reports the antiemetic effect of metoclopramide in patients receiving DDP.

MATERIALS AND METHODS

During a phase II study, 35 patients with histologically proven squamous cell carcinoma received combination chemotherapy that consisted of DDP, bleomycin, and high-dose methotrexate with citrovorum factor rescue. Thirty-three of these patients were diagnosed as having stage III or IV (M_0) carcinoma of the head and neck, one had carcinoma of the cervix uteri, and one had carcinoma of the esophagus. Most of these patients received one course of treatment; six, however, received two courses. Therefore, a total of 41 treatment courses were given. None of these patients have had any clinical or radiologic evidence of neurologic or gastrointestinal involvement.

DDP was given at a dose of 100 mg/m² iv over a 6-hour period on Day 1 of each course. This was preceded and followed by iv hydration.

During the first 28 courses of this regimen, severe nausea and vomiting were observed in 21 cases during the administration of DDP. It was also obvious that such nausea and vomiting occurred 3½–4 hours after the initiation of DDP therapy and lasted for 6–24 hours. Occasionally, vomiting persisted for 48–72 hours. In an attempt to control such vomiting, various antiemetic drugs were given parenterally and repeatedly prior to the initial vomiting and during the ensuing hours, including Thorazine, Compazine, Tigan, and Emeticon with no significant benefits. The next 13 courses of the same chemotherapy regimen were administered in the same fashion, but metoclopramide was administered in a single dose of two tablets orally (total, 20 mg) 3 hours after the initiation of DDP therapy.

RESULTS

The results have been expressed by both number of patients and number of courses in table 1. No neurologic or gastrointestinal side effects were noted. There was neither an increase in the bone marrow suppression nor any renal toxic effects with the addition of metoclopramide to the chemotherapy program.

Six patients received two courses of chemotherapy. Four of these patients received the combination chemotherapy without metoclopramide in either course and all vomited during both courses. Another patient received metoclopramide during each chemotherapy course and did not vomit. The last patient received his first course of chemotherapy without metoclopramide and vomited; however, when he received his second course of chemotherapy with metoclopramide, he did not experience nausea and did not vomit.

TABLE 1.—Effect of metoclopramide on DDP-induced vomiting

	Patients	Courses
Total No.	35	41
No. with vomiting/ No. *without* metoclopramide	17/24 (71%)	21/28 (75%)
No. with vomiting/ No. *with* metoclopramide	1/11 (9%)	1/13 (8%)

DISCUSSION

One of the common side effects of DDP is severe nausea and vomiting that, in our experience, could not be controlled by a variety of antiemetic agents. The administration of these antiemetic agents parenterally prior to and during DDP administration could have resulted in 25% control, while a single 20-mg oral dose of metoclopramide 3 hours after the initiation of DDP therapy gave 92% antiemetic control. The above results in this pilot study could indicate that metoclopramide may be an effective agent in controlling DDP-induced vomiting. Repeated administration of metoclopramide is being tried now, for even better control of vomiting, before initiating a prospective, randomized controlled study. This is because none of the mild self-limiting side effects of the drug were noted in our patients, and there was no evidence that metoclopramide increased the cytotoxic effect on other targets such as the bone marrow or the kidneys.

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Chemotherapy for Malignant Mesothelioma With Adriamycin and Continuous Infusion of 5-Azacytidine[1,2]

The incidence of malignant mesothelioma is rising (1). Little progress has been achieved in the treatment of this neoplasm by surgery and/or radiotherapy, and few chemotherapeutic studies have been conducted (1). Adriamycin is one of the most effective single agents (1). A phase I study of 5-azacytidine given twice weekly by iv injection has shown activity in two of three patients with mesothelioma (2). Although all responses were categorized as "questionable," one of these two patients responded for 9 months. It seemed logical to combine these two agents, especially since they showed synergism in the treatment of P388 leukemia in mice (3). The use of 5-azacytidine by continuous infusion has the advantage of minimizing gastrointestinal toxic effects and achieving more sustained plasma levels (4).

Eight patients with malignant mesothelioma have been studied. A diagnosis was established in each patient by thoracotomy and open surgical biopsy. All patients had evidence of progressive disease. Four patients had prior exposure to asbestos. Previous therapy included partial decortication in five patients, radiotherapy to the involved hemithorax in three patients, chemotherapy with other agents in two patients, and immunotherapy with bacillus Calmette-Guérin (BCG) in one patient. Informed consent was obtained from each patient. Pretherapy workup included chest roentgenogram, liver, bone, and brain scans, and cardiac and abdominal sonograms in all patients, and laparoscopy in two patients. All patients had normal cardiac, hepatic, and renal functions. Each cycle of chemotherapy included adriamycin given at a dose of 25 mg/m²/day by iv bolus from Day 1 to 3, and 5-azacytidine given at a dose of 120 mg/m²/day by continuous iv infusion in Ringer's lactate from Day 1 to 5, using a Volumetric Infusion Pump (McGaw Laboratories, Irvine, Calif). Fresh solutions were prepared every 4 hours and dissolved in < 100 ml of Ringer's lactate to avoid fluid overload. Courses were repeated every 4 weeks provided that there were no residual toxic effects (wbc count $\geq 4000/\mu l$ and platelet count $\geq 150{,}000/\mu l$).

Results are summarized in table 1. Patient 1 developed bilateral pleural effusion in October 1975, which was treated by thoracocentesis on the right and instillation of talc on the left. Massive ascites developed from June to October 1976. An abdominal sonogram revealed ascites and peritoneal thickening. Chemotherapy was begun after paracentesis. Complete remission with normalization of abdominal sonogram occurred in 4 months without further tapping. Patient 2 developed right pleural effusion in September 1973, which was treated by partial decortication and radiotherapy. In January 1976, a second thoracotomy was performed because of increasing dyspnea and the appearance of diffuse reticular nodular infiltrates in both lung fields. Pleural and pulmonary biopsies revealed persist-

[1] Supported in part by Public Health Service grant CA-15936 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare, and by grants from the International Association of Heat and Frost Insulators and Asbestos Workers and the Johns Manville Corp.

[2] 5-Azacytidine was supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, NCI, Bethesda, Md.

TABLE 1.—Patient characteristics, response, and survival

Patient No.	Sex/age	Extent of mesothelioma	No. of courses	Response and duration (mos)	Survival (mos) from— 1st treatment	Survival (mos) from— Diagnosis
1	F/59	Peritoneum and pleural bilateral	14	Complete (18+)	20+	32+
2	M/47	Right pleura, pulmonary bilateral, and subcutaneous tissue	14	Complete (18+)	20+	56+
3	M/62	Right pleura	5	Stable (5)	6	15
4	M/43	Right pleura and pericardium	4	Stable (4)	13+	25+
5	F/53	Right pleura and subcutaneous tissue	3	Progression	5	16
6	M/61	Right pleura	3	Progression	5	12
7	M/61	Right pleura, peritoneum, and subcutaneous tissue	2	Progression	2	11
8	M/61	Right pleura	2	Progression	3	7

ence of mesothelioma in the pleura, with interstitial and alveolar spread. Pathologic material is detailed in another report (5). The patient was treated elsewhere with prednisone, intradermal BCG, and laetrile without improvement. Chemotherapy was begun in October 1976, with complete regression of pleural and pulmonary disease seen on chest roentgenograms after four cycles. In both patients, adriamycin was discontinued after seven cycles (total dose, 525 mg/m^2) and replaced by cyclophosphamide given at a dose of 200 mg/m^2/day by iv bolus from Day 1 to 3.

Toxicity was moderate. Alopecia was universal but nausea and vomiting were minimal. Leukopenia (wbc count $< 3000/\mu l$) was seen in three patients and was reversible, with nadirs of 200, 2100, and 2600 cells/μl respectively, between Days 10 and 18 after the onset of treatment. Only one patient developed thrombocytopenia (26,000 platelets/μ l) which was reversible. Transient lethargy occurred in two patients. Local infiltration of 5-azacytidine with pain and erythema occurred in two patients. Mild pulmonary edema was seen once and was then avoided by using a minimal amount of Ringer's lactate ($\leq$ 100 ml every 4 hours).

Randomized trials will be undertaken in order to compare the present regimen with adriamycin alone and 5-azacytidine alone.

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Phase II Trial of Bleomycin in Bilharzial Bladder Cancer

Cancer of the bladder is the most common malignancy in Egypt, and almost always occurs in the setting of chronic bilharziasis. Bilharzial bladder cancer differs from that in the nonbilharzial bladder in several important respects: (*a*) its histology is predominately squamous cell rather than transitional cell; (*b*) its mortality is due to local complications rather than to distant metastases; (*c*) it rarely arises from the trigone; and (*d*) it is a disease predominately of middle-aged men (1).

Previous chemotherapeutic trials have shown methotrexate and VM-26 to be inactive. Hexamethylmelamine in doses of 8 mg/kg/day elicited partial responses in ten of 26 patients (2). Because of the activity of bleomycin in squamous cell cancer of other sites, this antibiotic was selected for a phase II trial in bilharzial bladder cancer in Egypt (3).

Twenty-one patients with UICC stage T_4 biopsy-proven bladder cancer met the following entry criteria: BUN < 40 mg/100 ml; serum creatinine < 2.5 mg/100 ml; wbc count $> 4000/mm^3$; platelet count $> 100{,}000/mm^3$; and expected survival of ≥ 8 weeks. Nineteen of the 21 patients had squamous cell cancer; there was one case each of anaplastic carcinoma and adenocarcinoma. No patient had received prior surgery, radiotherapy, or chemotherapy.

Bleomycin (15 units im) was administered daily $\times$ 3 and repeated at 3-week intervals until there was evidence of progressive disease. Three patients received one course, eight patients received two courses, seven patients received three courses, and three patients received four courses of bleomycin.

No patient had an objective reduction of tumor size. Toxicity was mild: one patient developed mild skin hyperpigmentation and no patients developed pulmonary or hematologic toxicity.

This trial shows that bleomycin in this dose and schedule is inactive in advanced bilharzial bladder cancer. It is of interest that both bleomycin and methotrexate, while active in squamous cell cancer of the head and neck, are inactive in squamous cell cancer of the bilharzial bladder.

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Acute Leukemia After Prolonged Immunosuppressive Therapy for Glomerulonephritis

Some recent papers have reported evidence of carcinogenic activity of alkylating agents in man; for instance, this has been suspected in the development of leukemia after treatment of ovarian cancer (1). There have also been isolated reports that such agents may induce malignancies after treatment of nonmalignant disorders such as nephritis, collagen diseases, and rheumatoid arthritis (2,3). We would like to emphasize the potential hazards involved in using these agents in the treatment of nonneoplastic disease in a report of two patients who developed acute leukemia following immunosuppressive therapy with alkylating agents for nonmalignant conditions.

CASE REPORTS

Patient 1

A 15-year-old white boy first presented in 1967 with acute glomerulonephritis following a streptococcal infection. He received chlorambucil from March 1970 to May 1973 in a daily dose of 4–12 mg and a total dose of 8.9 g. This treatment was interrupted in 1973 because of leukopenia. Thereafter, he received indomethacin for 15 days (100 mg daily) and dipyridamole. In May 1976 he was transferred to a kidney unit for marked weakness. His peripheral blood cell count was: 7.5 g/100 ml, hemoglobin; 1800 leukocytes/mm^3; and 57% mononucleated cells. He was transferred to our department where a bone marrow study showed 90% leukoblast cells leading to the diagnosis of acute myelomonocytic leukemia with monocytic predominance. He was treated with daunorubicin and cytosine arabinoside without success. He died 1 month later with blastic aplasia and infection.

Patient 2

A 56-year-old white man was treated for nephrosis with corticosteroids from March 1968 to March 1972. At that time, the failure of this treatment was suspected due to extensive histologic changes observed on kidney biopsy with proliferative glomerulonephritis and glomerular hyalinization. He received indomethacin, 100 mg/day for 1 month, and chlorambucil, 4–8 mg/day for 2 years with a total dose of 4.32 g. His condition improved, and treatment was discontinued in 1974. In May of 1974 he was referred to the general hospital in Avignon (Dr. P. Souteyrand) with complaints of general weakness. The hemogram showed a hemoglobin level of 11 g/100 ml, a leukocyte count of 3200/mm^3, and a platelet count of 80,000/mm^3. A bone marrow trephine biopsy showed myeloblastic leukemia with hypoplastic marrow. He died on November 4 with a leukocyte count of 600/mm^3 and 83% myeloblast cells.

DISCUSSION

Both patients in this report were treated with immunosuppressive chemotherapy for renal disease. They also received small doses of indomethacin (1 g and 3 g) which is known to suppress the bone marrow and cause agranulocytosis when given over a long period of time or in large doses. The duration of the exposure to immunosuppressive agents (chlorambucil) was 3 years in the first patient with a total dose of 8.9 g, and 2 years in the second with a total dose of 4.32 g. The latent period from onset of chemotherapy to leukemia was 6 years in the first patient and 4 years in the second.

These findings seem of special interest in view of recent reports (1,4) concerning increased risk of development of acute leukemia in patients treated for ovarian tumors. Animal experimentation has shown the carcinogenic potential of alkylating agents commonly used in chemotherapy, but there have been only a few cases involving treatment of nonmalignant diseases with such agents and which ended in acute leukemia or, sometimes, secondary neoplasm. The latent period of 4 and 6 years is consistent with that predicted in animal studies and in previous case reports of second malignancies in cancer patients (5). Thus, in the treatment of patients with renal diseases, the risk of development of secondary neoplasm is likely to become a major consideration when one uses immunosuppressive

drugs. The changes in the bone marrow (dysmyelopoiesis) seem to be an indication of an evolution towards acute leukemia.

These case reports support the earlier editorial comment (6) that "the long-term use of antineoplastic drugs, and particularly alkylating agents in patients with nonmalignant disease, deserves careful reassessment and prospective evaluation of the risk of carcinogenesis, . . ."

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March 30, 1978

ANNOUNCEMENTS

Conference on "Combined Modalities: Chemotherapy/Radiotherapy"

Hilton Head, South Carolina, November 15–18, 1978

The Committee for Radiation Oncology Studies, in conjunction with members of the Radiation Study Section and leading oncologists and with the support of the National Cancer Institute, is sponsoring a conference on the interaction of chemotherapy and radiotherapy. The meeting will focus on experimental results of laboratory and clinical studies of the interaction of selected cancer chemotherapeutic agents and radiation. The emphasis will be on the response of both normal and tumor tissues.

Attendance will be limited to people working in the field, certain invited speakers and participants, and those investigators who submit abstracts that are accepted by the organizing committee. It is intended to be a workshop-type meeting and abstracts will be presented either in open session or as poster-scientific exhibit sessions.

The research to be reported will include pharmacology, biochemistry, and basic chemical effects of the specific compounds, in vitro results with interaction of drug and radiation, in vivo normal tissue response to drug and radiation, and current clinical trial information. The following drugs are of special interest: bleomycin, adriamycin, cyclophosphamide, *cis*-platinum, and nitrosoureas. Papers on other agents will be considered for a session on other interesting and new agents. The organizing committee also invites nominations of young investigators who will be supported for travel to the meeting and who will not necessarily be presenting a paper.

For further details on the meeting write to: Theodore L. Phillips, MD, Chairman, Organizing Committee, Division of Radiation Oncology, University of California, M-330, San Francisco, Calif 94143.

23rd Annual Clinical Conference on "Cancer of the Genitourinary Tract"

Houston, Texas, November 2–3, 1978

The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute in Houston, announces the 1978 Clinical Conference on "Cancer of the Genitourinary Tract" to be held at the Shamrock Hilton Hotel, Houston, Texas, November 2–3, 1978. This Conference will focus on issues of diagnosis, staging, and therapy as they relate to the critical and sometimes controversial therapies of the multiple stages of urologic malignancies. The Conference will be jointly sponsored by The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute and the American Cancer Society, Texas Division, Inc. It will provide practicing primary care physicians, oncologists, and urologists with critical issues at the moving edge of therapeutic investigation on urologic malignancies. An opportunity will be provided for speakers and registrants to exchange information at the conclusion of each session. There will be a registration fee.

This medical-education offering meets the criteria for 14 hours in Category I of the Physician's Recognition Award of the American Medical Association. This program is also acceptable for 14 prescribed hours by the American Academy of Family Physicians.

For additional information write: George R. Blumenschein, MD, Office of Education, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston, Tex 77030.

Seminars on Oncology at Roswell Park Memorial Institute

Buffalo, New York

There will be a seminar on "Progress in Head and Neck Surgery" on November 9, 1978, organized by the Department of Head and Neck Surgery at Roswell Park Memorial Institute. The Chairperson is Dr. Donald Shedd.

There will be a $10.00 registration fee. *For further information contact:* Ms. Claudia Lee, Cancer Control, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

Conference on "Inorganic and Nutritional Aspects of Cancer"

La Jolla, California, January 3–5, 1979

The International Association of Bioinorganic Scientists, Inc, will hold its second conference (Inorganic and Nutritional Aspects of Cancer) on January 3–5, 1979, at the La Jolla Village Inn, La Jolla, California. The extraordinary success of the first conference held on January 3–5, 1977 (*see*: Bioinorganic Chemistry 7:359–365, 1977) has stimulated wide interest in this new area of research. Current activity in this field mandates a second conference on the same topic(s) covering: cancer epidemiology, metal carcinogenesis, theoretic aspects of carcinogenesis, metal complexes as anticancer drugs, occupational cancers, trace mineral metabolism, and cancer prevention, etc.

The conference speakers have already been elected. The conference is open for attendance by I.A.B.S. members as well as nonmembers. Attendance is limited and advanced registration is required. The conference fee for early registration is $75.00 for I.A.B.S. members and $100.00 for nonmembers (registration at desk is $120.00).

For further information or registration write to: G. N. Schrauzer, Cancer Conference, Department of Chemistry, University of California at San Diego, Revelle College, La Jolla, Calif 92093.

Third Annual Symposium on Rational Therapeutics

Costa Mesa, California, September 23 and 24, 1978

The Third Annual Symposium on Rational Therapeutics will be offered this September 23rd and 24th, Saturday and Sunday, 1978, by the Department of Pharmaceutical Services, City of Hope National Medical Center in Duarte, California. This advanced symposium has been designed to provide each participating member of the oncology team with the latest, readily usable information on the chemotherapeutic management of the cancer patient. In addition, the rational use of hyperalimentation in the oncology patient will be reviewed. A basic comprehension of malignant disease and its treatment is prerequisite.

This unique program will feature: (1) complications of the cancer chemotherapeutic agents adriamycin and cyclophosphamide, (2) clinical applications of chemotherapeutic agents *cis*-dichlorodiammineplatinum(II) and high-dose methotrexate, (3) applied pharmacokinetics in cancer chemotherapy of antineoplastics and high-dose methotrexate, (4) hyperalimentation and cancer, and (5) a seminar on breast cancer including the latest information regarding tamoxifen and aminogluthethimide chemotherapy. Each participant will have the opportunity to participate in three seminars to cover currently relevant areas of interest.

The faculty will include such speakers as, Stephen K. Carter, MD, Mitchell V. Kaminisky, MD, William Evans, PhD, Lawrence Einhorn, MD, Janet Maiuro, MS, and Karl Blume, MD. Twelve hours of accredited continuing education hours have been applied for from the California State Board of Pharmacy and from the California State Board of Nursing. The fee for the 2-day seminar will be $80.00. The program site is the South Coast Plaza Hotel, Costa Mesa, located in Southern California. *Further information can be obtained by contacting:* Thomas E. Saito, PhD, Program Chairman, Department of Pharmaceutical Services, City of Hope National Medical Center, 1500 E Duarte Rd, Duarte, Calif 91010.

National Cancer Institute Conference on *cis*-Platinum and Testicular Cancer

Washington, DC, September 21–22, 1978

A conference open to all physicians with a major interest in cancer treatment research will be held at the Shoreham Americana, Washington, DC, September 21–22, 1978. On the first day, the conference will review experimental and clinical data obtained with *cis*-platinum compounds and on the second day (morning only) the conference will deal with all aspects of testicular cancer, including *cis*-platinum-containing chemotherapy. *For information please write to:* Dr. Franco Muggia, Division of Cancer Treatment, National Cancer Institute, Bldg 37, Rm 6A17, Bethesda, Md 20014.

Fellowships in Medical Oncology: Providence Hospital

Applications are now being accepted for first and second year fellowships in medical oncology for January 1979 and June 1979 at Providence Hospital, a 458-bed teaching community hospital and member institution of the Southwest Oncology Group. The fellowship provides an opportunity for total and comprehensive diagnosis, treatment, and management of cancer patients. A basic science research laboratory augments clinical training.

Board eligible or certified candidates must have a minimum of 3 years in an approved internal medicine residency.

Send informative letter and curriculum vitae to: **Clarence B. Vaughn, MD, PhD, Medical Director, Oncology Service, Providence Hospital, 16001 West Nine Mile Rd, Southfield, Mich 48075 [telephone (313) 424-3341].**

Fellowships in Medical Oncology: Baystate Medical Center

Applications are now being accepted for fellowships in medical oncology at Baystate Medical Center beginning July 1979 and July 1980.

Located in Springfield, Massachusetts, this 1036–bed Tufts-affiliated institution offers a newly integrated oncology training program incorporating both Wesson Memorial and Springfield Hospital/Wesson Women's Units. The staff includes seven full-time hematologist/oncologists and four radiation therapists as well as practicing community oncologists.

Designed to prepare trainees for subspecialty boards in oncology, it offers clinical and clinical research activities in cancer chemotherapy, clinical pharmacology, and immunotherapy. Elective rotations in hematology, radiation therapy, and pathology are available. It includes extensive interdisciplinary emphasis on patient management and participation in national cooperative groups to aid in the management of patients with solid tumors and hematologic malignancies. An optional third year for combined hematology/oncology certification is available.

Prerequisites include 2 years of approved medical residency and American Board of Internal Medicine eligibility.

Send informative letter and curriculum vitae to: **Ronald C. DeConti, MD, Director of Oncology, Baystate Medical Center, Springfield Hospital/Wesson Women's Unit, 759 Chestnut St, Springfield, Mass 01107.**

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